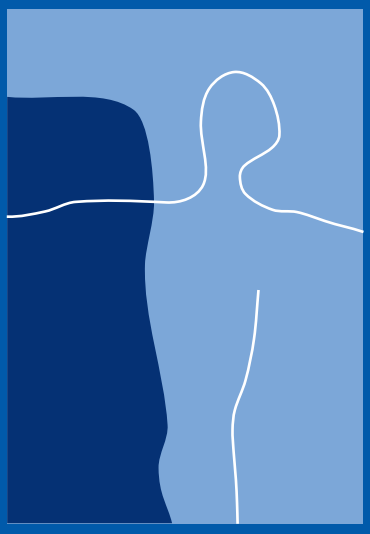

Acta CHIRURGICA Latviensis

2011 (11)

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Contents

ORIGINAL ARTICLE

Detection of Large Deletions and Duplications in Moderate Risk Breast Cancer Susceptibility Genes in Breast Cancer Patients Negative for the *BRCA1* and *BRCA2* Mutations

Dagnija Kalniete*, **, Arvīds Irmejs*, **, Ilze Štrumfa*, **, Jekaterina Žestkova*, **, Karīna Aksenoka *, **, Jānis Gardovskis*, **, Edvīns Miklaševičs*, **
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Summary
Introduction. Breast cancer is the most frequently diagnosed malignancy among woman in Latvia. Approximately 40% of all hereditary breast cancer cases can be explained due to the point mutations in the *BRCA1* and *BRCA2* genes. It is estimated that more than 10% of breast cancer predisposing mutations are large genomic rearrangements such as large deletions and duplications.
Aim of the study. The aim of the study was to determine a large deletions, or duplications in the moderate risk breast cancer susceptibility genes in breast cancer patients negative for the point mutations in the *BRCA1* and *BRCA2* genes.
Materials and methods. Study group consisted of 23 breast cancer patients negative for the 4153delA, 5382insC and 873delG, 886delTG mutations in the *BRCA1* and *BRCA2* genes, respectively. Multiplex ligation-dependent probe amplification was performed for the detection of large genomic deletions and duplications.
Results. Large genomic deletions were detected in the two cases. In one case was determined a large genomic deletion in the *CHEK2* gene spanning exons 9 and 10. In the second case exons 2 and 5 in the *CHEK2* gene and exons 29 and 46 in the *ATM* gene were deleted.
Conclusions. Large genomic deletions in the moderate risk breast cancer susceptibility genes can be found in Latvia. For the first time, a large genomic *CHEK2* gene deletion spanning exons 9 and 10 has been detected in Latvia.
Key Words: breast cancer, moderate risk breast cancer susceptibility genes, multiplex ligation-dependent probe amplification.

INTRODUCTION

Breast cancer is the most frequently diagnosed malignancy and leading cause of cancer death among women worldwide. It was estimated that approximately 1.38 million new cases were diagnosed and 558.400 females died from breast cancer in 2008 (Ahmed *et al.*, 2011). Breast cancer is the most common form of malignancy among female with approximately 1000 new cases diagnosed yearly in Latvia (Central Statistical Office of Latvia). The two major breast cancer susceptibility genes, *BRCA1* and *BRCA2,* are responsible for about 4% of all breast cancer cases in Latvia (Gardovskis *et al.*, 2009; Žestkova *et al.*, 2010) In the 3% of all breast cancer cases are found 4153delA, 5382insC mutations in the *BRCA1* gene and in the 1% of all breast cancer cases are found 886delTG and 873delG mutations in the *BRCA2* gene (Gardovskis *et al.*, 2009; Žestkova *et al.*, 2010). The *BRCA1* and *BRCA2* genes are responsible for about 30% of hereditary breast cancer cases worldwide and for about more than 40% in Latvia (Claus *et al.*, 1996, Gardovskis *et al.*, 2009). There is a strong association between *BRCA1* mutation status and morphology of the breast cancer. Approximately in the 80% of patients with the germ-line mutations in the *BRCA1* gene are found triple negative (TN) breast cancers (Spearman *et al.*, 2008). Triple negative breast cancers are attributed to the ER-negative, progesterone-receptor (PR)-negative and HER2-negative tumors (Perou *et al.*, 2000). Numerous studies have shown that large genomic rearrangements such as large genomic deletions or duplications are associated with the development of variety different hereditary conditions. For the most of the hereditary conditions partial gene deletions or duplications accounts for less than 10 % of all disease causing mutations and in some of the conditions it is 30% or more (Schouten *et al.*, 2002; Taylor *et al.*, 2003; Aretz *et al.*, 2007; Kanno *et al.*, 2007; Redeker *et al.*, 2008). Deletions or duplications of one or more exons of *BRCA1* and *BRCA2* genes predispose carriers to breast cancer. The prevalence of large genomic deletions and duplications in the *BRCA1* genes varies within different population but the overall rate is more than 10% and in the some of the populations even reaching almost 30% (Hartmann *et al.*, 2004; Pylkas *et al.*, 2008; Smith *et al.*, 2011; Hogervorst *et al.*, 2003). The large genomic deletions and duplications in the moderate risk breast cancer genes are studied less therefore in this study we focused our attention to moderate risk breast cancer susceptibility genes. *ATM* gene is a large gene containing 66 exons spanning approximately 150 kb of genomic DNA and over 400 mutations have been identified (Uziel *et al.*, 1996; Campbell et al., 2003). Due to its relatively

large size mutation detection is labor-intensive and times consuming thus a large proportion of mutations remains undetected. *CHEK2* gene spans approximately 50 kilobases of genomic DNA and consists of 14 exons (Bartek *et al*., 2001). 1100delC, I157T, IVS2+IG>A and 5.4 kb deletion of the *CHEK2* gene has been associated with an icreased risk of breast cancer (Wu *et al*., 2001; Meijer-Heijboer *et al*., 2002; Kilpivaara *et al*., 2004, Cybulski *et al*., 2006).

AIM OF THE STUDY

The aim of this study was to determine a large genomic deletions or duplications in the moderate risk breast cancer susceptibility genes (*CHEK2* and *ATM*) in breast cancer patients negative for the 4153delA, 5382insC and 873delG, 886delTG mutations in the *BRCA1* and *BRCA2* genes, respectively.

MATERIALS AND METHODS

Study group consisted of 23 breast cancer patients negative for the 4153delA, 5382insC and 873delG, 886delTG mutations in the *BRCA1* and *BRCA2* genes, respectively. All breast cancer patients were hospitalized from 2006 to 2008 at the Pauls Stradins Clinical University Hospital and Oncology Center of Latvia. The ages of patients at the diagnosis of the disease were from 41 to 71 years. Four healthy control donors were used as reference samples. All individuals signed informed consent forms for participation in the research. The study was approved by the Ethics Committee of the Riga Stradins University.

Genomic DNA was extracted from 2 ml of peripheral blood samples by FlexiGene DNA Kit (250) (Qiagen, Dusseldorf, Germany) according to the manufacturer's instructions.

Multiplex Ligation Probe Dependent Amplification (MLPA) was done by MLPA kit P190 (MRC-Holland, Amsterdam, Netherlands) according to the instructions of the manufacturer. MLPA was performed in the TProfessional thermal cycler (Biometra, Gottingen, Germany). Fragments were separated on Applied Biosystems 3130 capillary genetic analyzer and analyzed by GeneMapper Software v4.0 (Applied Biosystems, Foster City, CA).

MLPA data were normalized using block normalization. The relative probe signals were obtained by dividing the peak area of each amplification product by the total peak area of only the reference probes in the probemix. Probe ratios were calculated by dividing the relative probe signals in the cancer samples by the average of the relative probe signals in the reference (healthy controls) samples. Probe ratios between 0.7-1.3 were defined as normal.

Multiplex PCR was used to confirm a large genomic deletion in the *CHEK2* gene spanning exons 9 and 10. Multiplex PCR was performed in the TProfessional thermal cycler (Biometra, Gottingen, Germany) using specific primers (Cybulski *et al*., 2007). The cycling conditions were 3 minutes at 95°C, followed by 10 cycles of 30 seconds at 95°C, 30 seconds at 65°C and 30 seconds at 72°C and 40 cycles of 30 seconds at 95°C, 30 seconds at 55°C and 30 seconds at 72°C and fallowed by 10 minutes at 72°C.

Sequencing by Applied Biosystems 3130 capillary genetic analyzer was performed to detect the range of the deleted fragment in the *CHEK2* gene spanning exons 9 and 10.

Data were analyzed by Sequencing Analysis v5.3.1. and SeqScape v2.6.

RESULTS

The immunohistochemistry data of 23 breast cancer cases were analyzed. Thirteen breast cancers were diagnosed with triple negative (TN) cancer morphology and ten breast cancers were diagnosed with HER2 3+ cancer morphology. From 13 TN breast cancers cases a hereditary breast cancer (HBC) was diagnosed in 2 cases, hereditary breast ovarian cancer (HBOC) was diagnosed in 1 case and as being suspicious for hereditary breast cancer (HBC susp.) were diagnosed 3 patients. From 10 HER2 3+ with HBOC was diagnosed 1 patient.

In two cases large genomic deletions were detected. In one case was found a large genomic deletion in the *CHEK2* gene spanning exons 9 and 10. In the second case exons 2 and 5 of the *CHEK2* gene and exons 29 and 46 of the *ATM* gene were deleted.

Patient E111 showed reduced probe ratios of exons 9 and 10 of the *CHEK2* gene. The values of the probe ratios of exons nine and ten were 0.43 and 0.36, respectively (Figure 1A). Probe ratios between 0.7 and 1.3 were defined as normal and probe ratio under 0.7 were defined as deletion. The capillary electrophoresis peak profiles of exons 9 and 10 showed more than two times lower peak profiles in the cancer sample (Figure 1B) than adjacent peak profiles in the reference sample (Figure 1C) thus implying to the deletion of the two sequential exons in the *CHEK2* gene. E111 was diagnoses with a triple negative breast cancer at age 51. E111 aunt was diagnosed with breast cancer at age 60 and she died at the same age. Patient has five siblings with unknown age and health status (Figure 2). DNA samples for the rest of the siblings were not available. All siblings refused to participate in the study. A large genomic deletion of exons 9 and 10 of the *CHEK2* gene was confirmed by multiplex PCR. Amplification product gave an additional 450 base pair fragment thus implying to large genomic deletion as was previously described (Cybulski *et al*., 2006). Sequencing results revealed that deletion break-point sites are at 39867 bp and 45262 bp positions at the *CHEK2* gene (NT_011520) and our estimated length of the deleted fragment is 5395 base pairs.

Patient E224 showed reduced probe ratios of exons 2 and 5 of the *CHEK2* gene and reduced probe ratios of exons 29 and 46 of the *ATM* gene. The probe ratio values of exons 2 and 5 of the *CHEK2* gene were 0.64 and 0.63, respectively. The values of the probe ratios of exons 29 and 46 of the *ATM* gene were 0.62 and 0.59, respectively (Figure 4A). Peak profiles of exons 2 and 5 were reduced when were compared with adjacent peak

profiles of the reference sample. The same trend was seen in the case of the *ATM* gene (Figure 4B and 4C). E224 was diagnosed with the HER2 3+ breast cancer at age 69 and she has three siblings with unknown age and health status. The DNA samples from siblings were not available.

DISCUSSION

This is the first report of a large genomic deletion in the *CHEK2* gene spanning exons 9 and 10 in Latvia. *CHEK2* gene is a tumor suppressor gene which initiates DNA repair after double-strand breaks and has been found to be a breast cancer predisposing gene in several populations (Matsuoka *et al*., 1987; Chehab *et al*., 2000). A large genomic deletion in the *CHEK2* gene has been previously reported and has been associated with the elevated risk of breast cancer (Walsh *et al*., 2006; Cybulski *et al*., 2007; Bogdanova *et al*., 2007). A deleterious *CHEK2* gene mutation is prevalent in Central and Eastern European populations. Initially deletion was found in 8 breast cancer patients from the Czech Republic and Slovakia and was considered to be a founder mutation for these particular populations (Walsh *et al*., 2006). Later, a large genomic deletion was found in the 1% of unselected breast cancer cases in Poland. Mutation was present in the 0.4% of the population and was associated with approximately twice the risk of breast cancer (Cybulski *et al*., 2007). Deletion has been also associated with the prostate cancer and is considered to be one of the most widespread protein-truncating mutations in the *CHEK2* gene in Poland (Cybulski *et al*., 2006). By our estimations, the length of the deleted fragment spanning exons 9 and 10 is 5395 base pairs and it is the same length as was found in Poland (Cybulski *et al*., 2006). As with Polish we have common 4153delA, 5382insC founder mutations in the *BRCA1* gene it is very likely that 5395 base pair deletion in the *CHEK2* gene is a founder mutation in Latvia (Gorski *et al*., 2000). It would be very important to verify the prevalence of large deletion in Latvian population and to estimate relative risk for breast and prostate cancers patients carrying particular large genomic deletion.

In second case exons 2 and 5 in the *CHEK2* gene and exons 29 and 46 in the *ATM* gene were found to be deleted. A protein kinase encoded by *ATM* gene is involved in mitogenic signal transduction, meiotic recombination, detection of DNA damage, and cell cycle control (Savitsky *et al*., 1995). Ataxia telangiectasia is an inherited condition characterized by progressive cerebella ataxia, oculomotor apraxia, frequent infections, immunodeficiency, sensitivity to ionizing radiation, and increased risk of malignancy (Jasper *et al*., 1998; Gatti *et al*., 1991). A female relatives being heterozygous for an *ATM* gene mutations in the families of individuals with ataxia telangiectasia have an approximately two to five fold increase in risk of breast cancer (Swift *et al*., 1987; Thompson *et al*., 2005; Renwick *et al*., 2006) A truncating variant Glu1978X of the *ATM* gene has been associated with a large increase in breast cancer risk (Ahmed *et al*., 2006; Renwick *et al*., 2006). Deletions of exons 29 and 46 of the *ATM* gene and exons 2 and 5 of the *CHEK2* gene have not been previously reported and ask for further research.

CONCLUSIONS

1. A large genomic deletions in the moderate risk (*CHEK2* and *ATM*) breast cancer susceptibility genes can be found in Latvia.
2. This is the first report of a large genomic deletion in the *CHEK2* gene spanning exons 9 and 10 in Latvia.

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Conflict of interest: None

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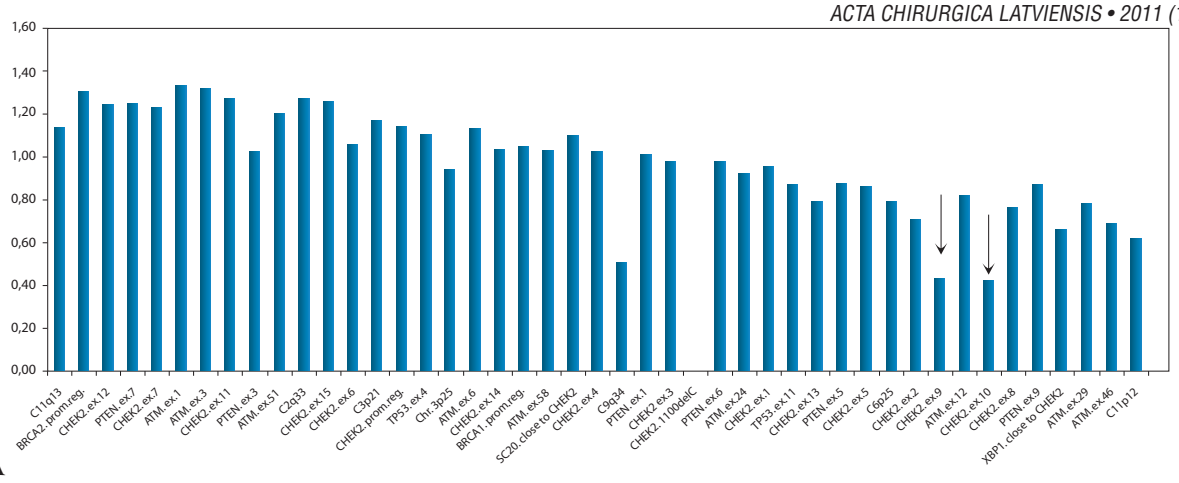
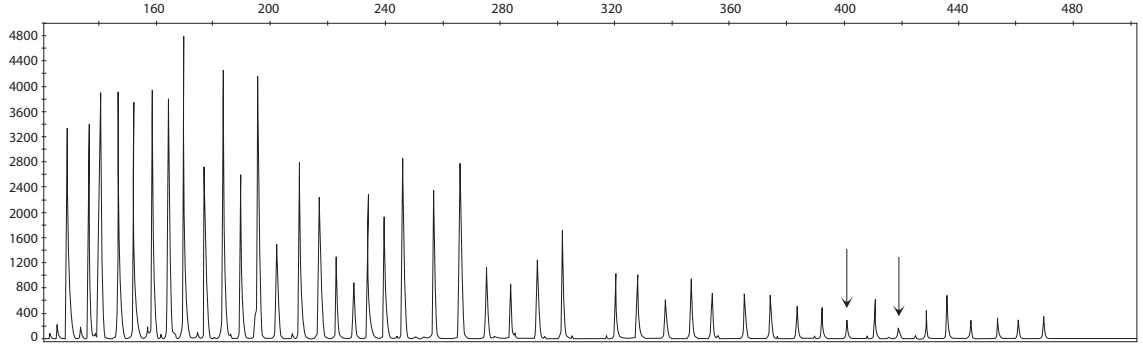


1A



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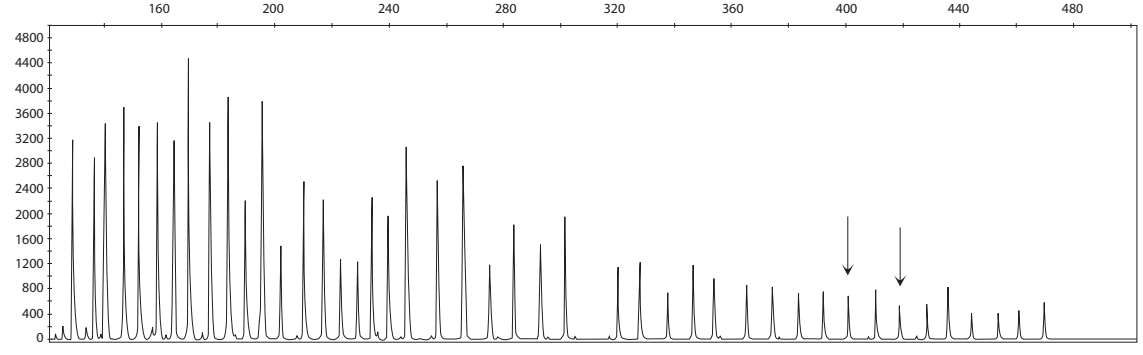


1C

Fig.1a, 1b, 1c. (1A) Probe ratios calculate for a breast cancer patient E111 with a large genomic deletion of the *CHEK2* gene spanning exons 9 and 10. (1B) MLPA electropherogram of a breast cancer patient E111 carrying a large genomic deletion in the *CHEK2* gene. MLPA electropherogram of a healthy control (1C). With arrows are indicated probes of exons 9 and 10 of the *CHEK2* gene.

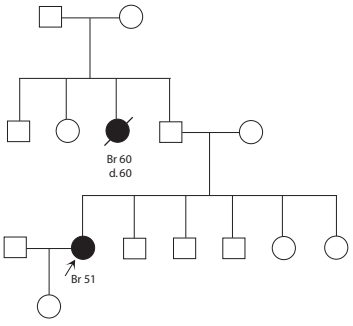


Fig. 2. Family pedigree of a breast cancer patient E111.

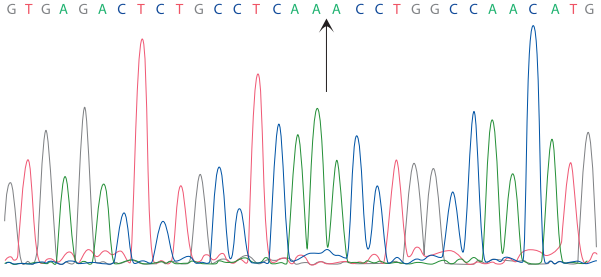
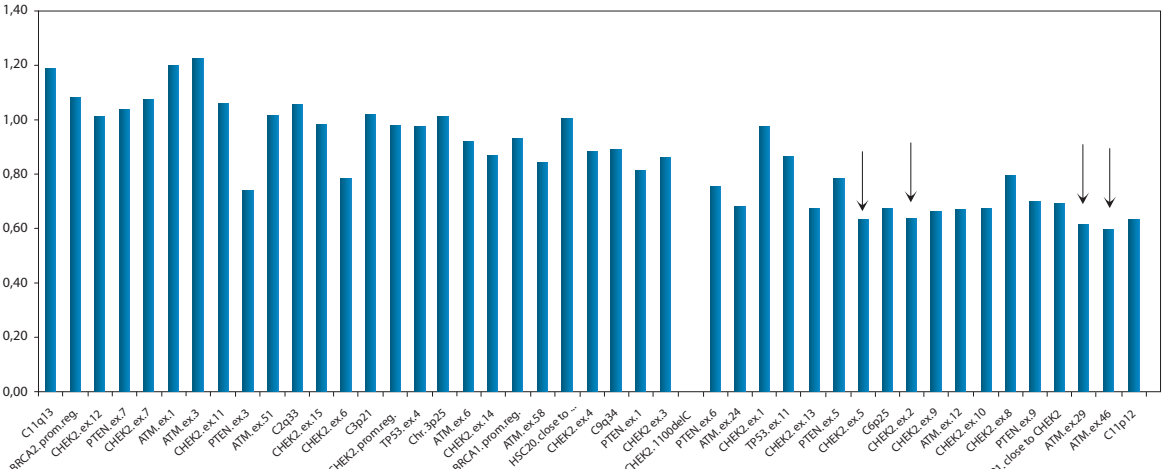
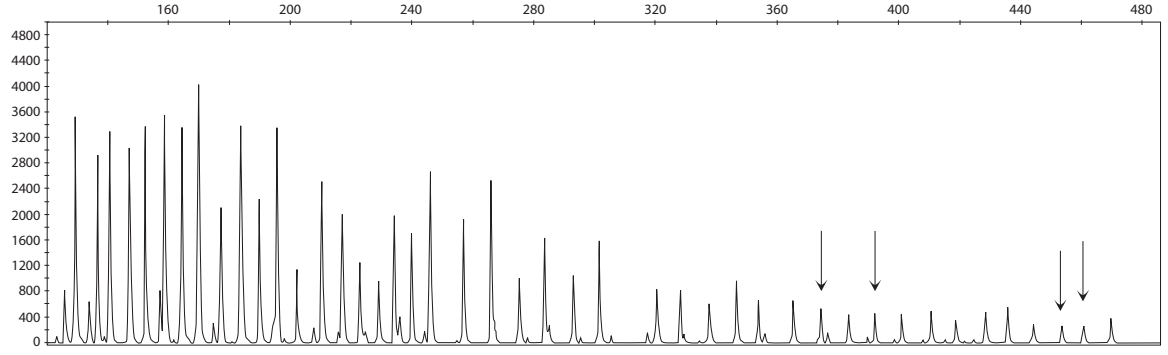


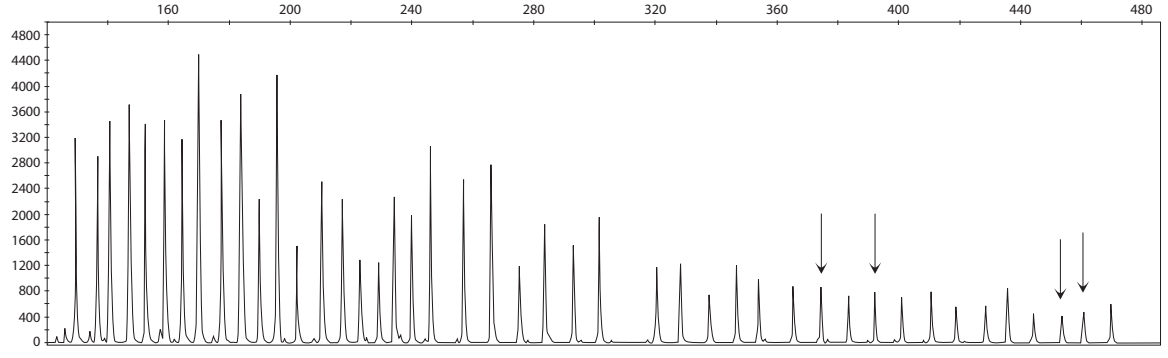
Fig. 3. Sequencing of a genomic breakpoint in a breast cancer patient E111 demonstrating a large deletion of exons 9-10 of the *CHEK2* gene.



4A



4B



4C

Fig. 4a, 4b, 4c. (4A) Probe ratios calculate for a breast cancer patient E224 with deleted exons 5 and 2 in the *CHEK2* gene and exons 29 and 46 in the *ATM* gene. (4B) MLPA electropherogram of a breast cancer patient harboring deleted exon 5 and exon 2 in the *CHEK2* gene and exons 29 and 46 in the *ATM* gene. MLPA electropherogram of a healthy control (4C). With arrows are indicated probes of exons 5 and 2 of the *CHEK2* gene and probes of exons 29 and 46 of the *ATM* gene.

ORIGINAL ARTICLE

Primary Breast Lymphoma: Clinical and Morphological Characteristics

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Summary
Introduction. Primary breast lymphoma constitutes only 0.004 – 0.5% of malignant breast tumours. The correct diagnostics of this rare neoplasm necessitates routine application of evidence-based diagnostic methods including immunohistochemistry (IHC).
The aim of the study was to detect the frequency of primary breast lymphoma among consecutive well-characterised breast tumours in Latvian patients and to provide the essential clinical, radiological and morphological characteristics.
Materials and Methods included review of the morphological data and routine IHC as well as clinical, laboratory and radiological findings in 474 consecutive patients diagnosed with malignant breast tumours.
Results: The frequency of primary breast lymphoma was 0.63% [95% confidence interval = 0.22 – 1.84%]. All cases were represented by diffuse large B-cell lymphoma. Rapid progression, unilateral localisation and no evidence of extra-mammary spread was characteristic. Occurrence in a male patient was also observed.
Conclusions: Breast lymphoma occurs in the local population with the incidence 0.63% of malignant breast tumours. The lesion retains significance for practising surgeon due to diagnostic pathway and occasional application of surgical treatment. Diffuse large B-cell lymphoma is the most frequent type of breast lymphoma. Immunohistochemical investigation is mandatory to identify breast lymphoma and perform reliable differential diagnosis with other high-grade malignant tumours.
Key words: breast tumour, extranodal lymphoma, breast lymphoma.

INTRODUCTION

Primary breast lymphoma is a rare disease (Neri *et al.*, 2008; Jeanneret-Sozzi *et al.*, 2008; Ryan *et al.*, 2008) accounting for 0.004 – 0.5% of malignant breast tumours (Jeanneret-Sozzi *et al.*, 2008). However, the entity is gaining more significance due to increasing frequency of extranodal non-Hodgkin's lymphomas. Also, larger retrospective (Ryan *et al.*, 2008) and at least some prospective (Aviles *et al.*, 2006) studies have been carried out providing more information about breast lymphoma. The largest series including 204 retrospectively analysed cases provided evidence that diffuse large B-cell lymphoma of the breast represents a distinct entity (Ryan *et al.*, 2008). However, the diagnostics of breast lymphoma is embarrassed by the rarity of the tumour.

The diagnostic criteria of primary breast lymphoma include the request for adequate pathologic diagnosis and close association between mammary tissue and lymphomatous infiltrate. Prior diagnosis of extramammary lymphoma and concurrent widespread disease except ipsilateral axillary lymph nodes must be excluded (Wiseman and Liao, 1972). Thus, the occurrence of primary breast lymphoma can be detected only by routine application of evidence-based diagnostic methods.

AIM OF THE STUDY

The aim of our sudy is to detect the frequency of primary breast lymphoma among consecutive well-characterised malignant breast tumours in local patients and to provide the essential clinical, radiological and morphological characteristics.

MATERIALS AND METHODS

Retrospective archive search was performed to identify consecutive patients who have been subjected to diagnostic breast core biopsy and/ or operation during the time period of 31 months (2009 – July 2011). The respective tissue materials were identified and reviewed by two researchers (AA, IS) in order to reach consensus conclusion. All the tissues have been fixed in 10% neutral buffered formalin, processed in vacuum infiltration processor Tissue-Tek® VIP™ 5 (Sakura Seiki Co., Ltd., Nagano, Japan), embedded by tissue embedding system TES 99 (Medite GmbH, Burgdorf, Germany) in paraplast (Diapath S.r.l., Belgamo, Italy), cut into 4 micron thick sections by microtome HM 360 (Microm, Waldorf, Germany), stained and investigated by protocol approach to identify the presence, histological type and characteristics of tumour. The tumours were diagnosed by criteria as described (Mills *et al.*, 2009). Immunohistochemical investigation was applied systematically. If malignancy was identified or suspected, breast cancer panel (Table 1) was applied. If the histogenesis of tumour was not clearly epithelial by the mentioned criteria (Mills *et al.*, 2009) and/or breast cancer panel yielded negative results only, the epithelial origin was investigated by cytokeratin AE1/ AE3. Anaplastic tumours were subjected to additional studies by panels for the diagnostics of metastatic versus primary breast cancer, melanoma and lymphoma (Table

1). For immunohistochemistry (IHC), the formalin-fixed, paraffin-embedded tissues were cut at 3 micron thick sections on electrostatic slides (Histobond, Marienfeld, Germany) and subjected to heat-induced epitope retrieval in TEG buffer at pH 9.0 in microwave oven 3x5 min. Panel of primary antibodies was employed (Table 1). Peroxidase-conjugated labelled streptavidin-biotin visualization system was applied for the detection of bound primary antibodies, followed by colour development by 3,3'-diaminobenzidine. All IHC reagents were produced by Dako, Glostrup, Denmark. Positive and negative controls were performed and reacted appropriately. The clinical histories were reviewed in order to obtain the age and sex characteristics, laboratory investigations and radiological data as well as the recommended treatment. Descriptive statistical analysis was performed. The 95% confidence intervals (CI) were calculated by CIA software (Altman *et al.*, 2000).

RESULTS

Three cases of primary breast lymphoma were identified among 474 consecutive malignant breast tumours over the time period of 31 months (2009 – July 2011). The frequency of breast lymphoma thus was estimated 0.63% [95% CI = 0.22 – 1.84%].

All patients, including 2 females and 1 male, were elderly (Table 2). The clinical data are reflected in Table 2. In all cases, local findings constituted the main basis of symptoms. The male patient had serious complaints due to large, painful, exulcerated breast mass. Another patient approached doctor due to palpable mass in the breast. Rapid growth was reported by both these patients. In the last patient, the tumour was discovered incidentally on mammogram although the lesion was palpable by objective investigation. The size of the lesions is shown in Table 2. The largest diameter of breast lymphomas, ranging 3.7 – 20.0 cm, exceeded the 95% confidence interval of the mean largest diameter of primary breast cancer among the investigated consecutive cases, namely 2.8 cm [95% CI = 2.4 – 3.2]. The diameter of epithelial breast malignancies diagnosed over the same time period ranged 0.1 – 19.5 cm.

In all cases, the tumours affected the left breast. There was no evidence of bilateral or multifocal mammary lesion by clinical examination (3/3), ultrasonography (3/3) and mammography (2/2 female patients). No lymph node involvement was found clinically or by ultrasonography. Ultrasonographically, the pathologic foci were characterised by high vascularity and indistinct borders.

The laboratory investigations yielded the following data. Only the patient with giant exulcerated tumour had abnormalities in blood cell composition, namely, leukocytosis with white blood cell count 22.6 x 10^9/L (reference interval 4.0 – 10.0); anaemia with red blood cell count 2.9 x 10^{12}/L(4.5 – 6.6) and thrombocytosis with platelet count 597 x 10^9L (150 – 400). The biochemical blood tests mainly showed the manifestations of other diseases. Elevated level of glycosylated haemoglobin 7.1% (reference interval less than 6.0), and blood glucose 11.9 mmol / L (3.3 – 5.9) was found in a patient with diabetes mellitus. The male patient, previously diagnosed with benign prostate hyperplasia and arterial hypertension, had elevated levels of prostate specific antigen 6.34 ng/mL (reference interval not exceeding 4.00) and creatinine 240 micromol/ L (53 – 113).

The histology (Fig.1-2) in all cases yielded high-grade diffuse large B-cell non-Hodgkin's lymphoma with positive expression of CD20 (Fig.3), CD79alpha and leukocyte common antigen. The proliferation fraction was invariably high (Fig.4) reaching 70.0 – 92.0%, mean 80.5%. The neoplastic cells were negative for cytokeratins AE1/AE3 (Fig.5), mammaglobin, oestrogen and progesterone receptors and CD30. High vascularity was consistent with the radiologic findings (Fig.6). Close association with mammary gland tissue was shown (Fig.7-8). Axillary lymph nodes were available for morphologic investigation in a single case and showed no malignancy. Diagnostic difficulties were present in all cases due to pseudoalveolar grouping of tumour cells, immaturity of lymphoma cells, presence of massive reactive polyclonal plasmocytosis and presence of fibrosis, probably due to pre-existent fibrous mastopathy. In all cases, IHC provided evidence-based substantiation of the diagnosis by showing unequivocal signs of histogenesis and monoclonality of tumour cells. During the same time period, 14.3% [95% CI = 11.5 – 17.8%] of breast core biopsies necessitated extended IHC beyond the scope of breast cancer panel due to high tumour grade.

The treatment was diverse. Chemoradiotherapy was advised for 2 patients. Lumpectomy with axillary lymph node dissection was performed in one patient. The patient with the largest tumour received palliative care only in order to lessen the previously uncontrolled pain and to take care of the exulceration. Subjective improvement was achieved. However, death of this patient ensured 14 days after he was discharged from the hospital.

DISCUSSION

Breast lymphoma is a rare breast tumour constituting 0.04 – 0.53 % of primary malignant breast tumours (Anavekar *et al.*, 2007; Jeanneret-Sozzi *et al.*, 2008), 1.7 – 2.2% of extranodal lymphomas and 0.38 – 0.7% of non-Hodgkin's lymphomas (Anavekar *et al.*, 2007). In our series including 474 consecutive malignant breast tumour cases, the 95% confidence interval of the frequency approaches the highest level.

In order to diagnose breast lymphoma, appropriate diagnostic tissue investigation is mandatory in accordance with the diagnostic criteria. It should be noted that the request for adequate diagnostics was initially included as the first criterion pointing towards cardinal importance. Our experience is in accordance with this as high grade primary breast cancer or metastatic tumours cannot be reliably distinguished from lymphoma by routine haematoxylin-eosin stains only. Systematic use of morphological diagnostic criteria

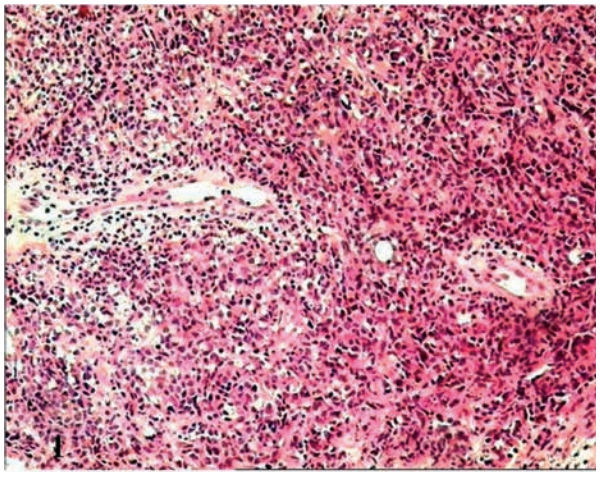

Fig. 1. The tissue structure of the tumour. Haematoxylin-eosin (HE), original magnification (OM) 100x.

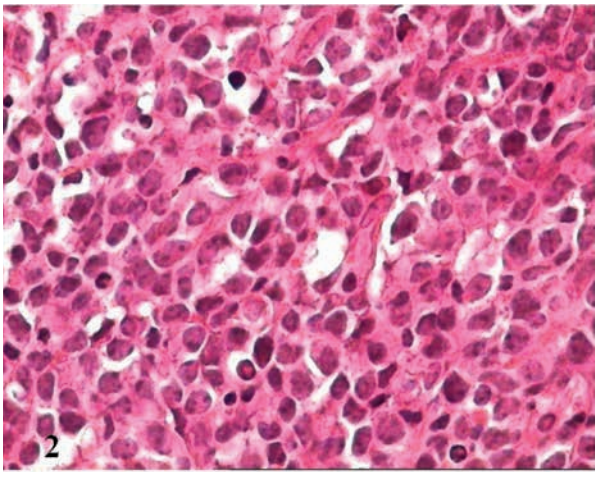

Fig. 2. Marked anaplasia in the neoplastic cells revealing high grade malignant tumour. HE, OM 400x.

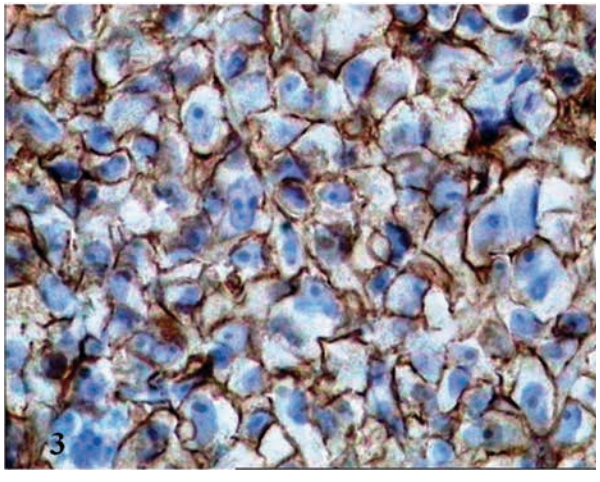

Fig. 3. Intense expression of CD20. Immunoperoxidase (IP), anti-CD20, OM 400x.

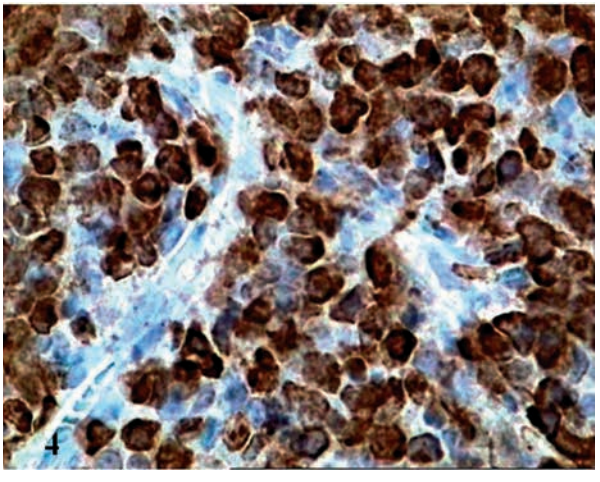

Fig. 4. High proliferative activity in the neoplastic cells. IP, anti-Ki-67, OM 400x.

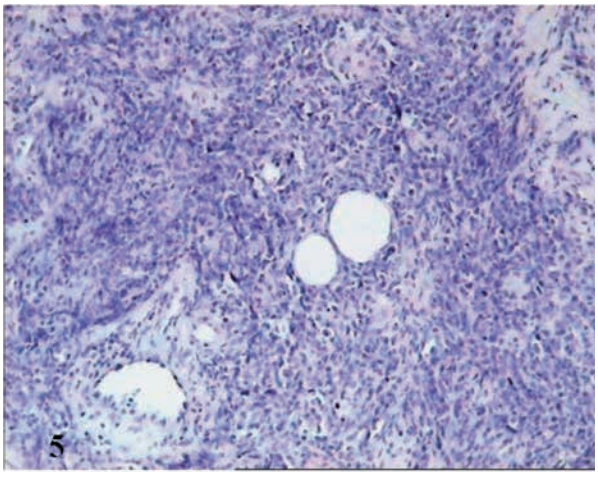

Fig. 5. Lack of cytokeratin in the tumour. Note also the widespread infiltrative growth. IP, anti-cytokeratin AE1/AE3, OM 100x.

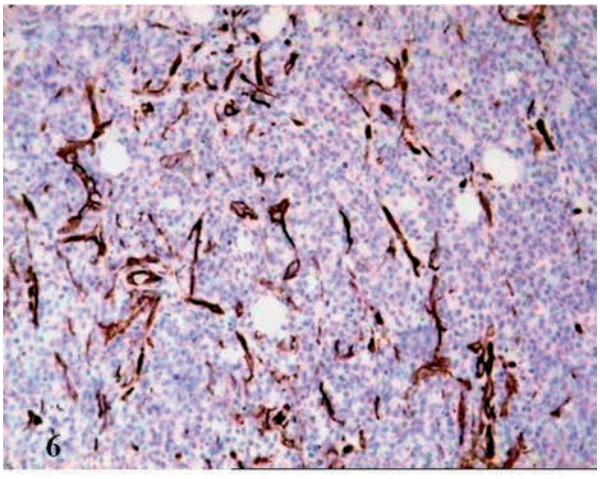

Fig. 6. High microvessel density corresponding to the high vascularity by ultrasonography. IP, anti-CD34, OM 100x.

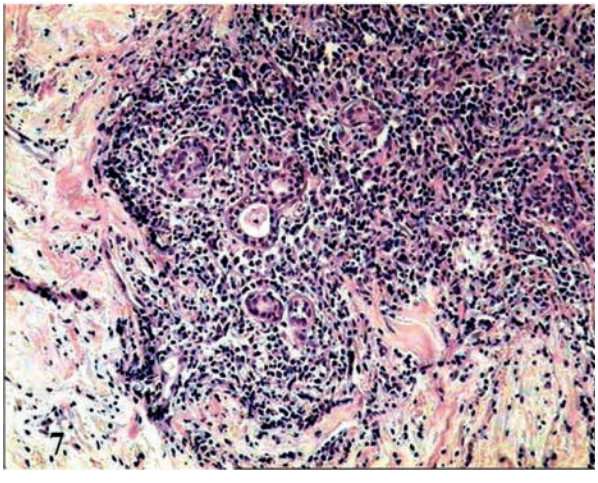

Fig. 7. Close association between the neoplastic infiltrate and mammary tissue. HE, OM 100x.

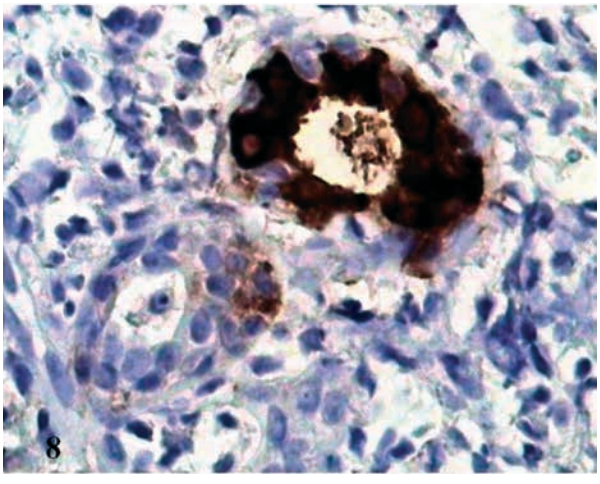

Fig. 8. Mammaglobin expression confirming the mammary origin of the entrapped acinus. IP, anti-mammaglobin, OM 400x.

Table 1. Logistics and technological details of the applied immunohistochemical investigation

Aim	Antibody	Clonality	Dilution	InT, min.	Indications
Characteristics of breast cancer (breast cancer panel)	Oestrogen receptor alpha	1D5	1:1	60	Routine
	Progesteron receptor	PgR636	1:1	60	Routine
	Ki-67	MIB-1	1:100	60	Routine
	E-cadherin	NCH-38	1:50	60	Routine
	Actin	HHF35	1:400	60	Routine
CUP panel	Cytokeratin	AE1/AE3	1:200	60	Epithelial origin
CUP panel	Mammaglobin	304-1A5	1:100	60	Primary origin of cancer in the breast
	TTF-1	8G7G3/1	1:100	60	Metastasis of pulmonary small cell cancer or adenocarcinoma
	CDX-2	DAK-CDX2	1:50	60	Metastatic colorectal cancer
	CD56	123C3	1:100	60	Neuroendocrine tumour
	Chromogranin A	Polyclonal	1:1000	60	Neuroendocrine tumour
Melanoma panel	S-100	Polyclonal	1:4000	60	Melanoma
	Melanosome	HMB-45	1:200	60	Melanoma
Lymphoma panel	LCA	2B11+PD7/26	1:200	60	Lymphoma
	CD20	L26	1:1000	60	Benign or malignant B-cells
	CD30	Ber-H2	1:50	60	Hodgkin's lymphoma, anaplastic lymphoma
	CD79alpha	JCB117	1:100	60	Benign or malignant B-cells
	CD3	Polyclonal	1:1	10	Benign or malignant T-cells
	Kappa light chains	Polyclonal	1:1	10	Differential diagnostics of lymphoma
	Lambda light chains	Polyclonal	1:1	10	Differential diagnostics of lymphoma

Abbreviation in Table: InT, incubation time; min., minutes; CUP, cancer of unknown primary origin

Table 2. The clinical and radiological characteristics of primary breast lymphoma

No.	Sex	Age	Side	Size, cm	Axillary lymph nodes		Tumour course	Other diseases
					Clinically	By US		
1	F	66	L	3.7x3.5x2.5	Not enlarged	Insignificant: 1.2x0.5 cm	Breast mass, known for 1 mo	Arterial hypertension Diabetes mellitus
2	F	82	L	6.0x5.8x3.0	Not enlarged	Insignificant: 1.1, 0.7 and 0.9 cm in diameter	Breast mass 3 mo, rapid growth	Surgically removed colloid goiter 3 years ago
3	M	86	L	20.0x15.0x15.0	Not enlarged	No evidence of lymph node pathology	Breast mass 6-8 mo Local pain 3 mo	Benign prostate hyperplasia Arterial hypertension

Abbreviations in the Table: US, ultrasonography; F, female; M, male; L, left (breast); mo, months

in combination with routine immunohistochemistry is mandatory both to make the diagnosis of breast lymphoma in individual case and to determine the frequency of lymphoma among breast lesions. As we have implemented protocol approach for the diagnostic investigation of all breast lesions, the data are reliable by the quality of diagnostics. Thus, the present article includes the first well-proved estimate of the incidence of breast lymphoma among malignant breast tumours in Latvia.

Coexistence of lymphoma with breast cancer is described (Arlen *et al.*, 2011). Both ductal (Arlen *et al.*, 2011) and lobular (Anavekar *et al.*, 2007) carcinoma have been found to affect breast simultaneously with lymphoma. The coexistence of breast cancer and lymphoma has been attributed to the pathogenetic role of mouse mammary tumour virus (Arlen *et al.*, 2011).

The simultaneous occurrence of epithelial and haematological malignant breast tumours points towards careful diagnostic examination of multifocal breast lesions. Encountering the possibility of different nature of breast lesions, tissue for diagnostic evaluation should be obtained from each lesion (Anavekar *et al.*, 2007; Arlen *et al.*, 2011). This is even more important in case if the first core biopsy yields lymphoma and non-surgical treatment approach is advocated. However, there were no bilateral or multifocal cases in our group. Surprisingly, all cases have occurred at the left side although some researchers have described more frequent development of breast lymphoma at the right side (Liu *et al.*, 2005; Jennings *et al.*, 2007; Neri *et al.*, 2008). This difference almost certainly can be attributed to small number of cases.

Breast lymphoma more frequently (up to 98 – 100% of cases) occurs in female (Ryan *et al.*, 2008; Yhim *et al.*, 2010). However, a case in male has been observed among our patients. This is very uncommon yet well-proved observation. Only 10 cases of breast lymphoma in male were published until 2010 (Avenia *et al.*, 2010). The average age of diffuse large B-cell breast lymphoma patients is 64 years with range 15 – 89 years (Ryan *et al.*, 2008). The mean reported tumour diameter is 4.0 cm, ranging 1 – 20 cm (Ryan *et al.*, 2008). The characteristics of our cases are in agreement with the published data.

All the identified cases were diffuse large B-cell lymphoma. Our findings are in accordance with the published data describing diffuse large B-cell lymphoma as the most frequent breast lymphoma type with the frequency 45 – 79% of breast lymphoma cases (Liu *et al.*, 2005; Jennings *et al.*, 2007; Anavekar *et al.*, 2007). The other reported types include MALT lymphoma, extranodal follicular and marginal zone B-cell lymphoma as well as heterogeneous group of T-cell non-Hodgkin's lymphoma (Anavekar *et al.*, 2007; Gualco *et al.*, 2009; Martinelli *et al.*, 2009; Avenia *et al.*, 2010).

Although in general haematological neoplasms can be successfully treated, the prognosis of primary breast lymphoma is considered serious. The reported mean overall survival of 8.0 years [95% CI = 6.5 – 10.9] is lower than in combined group of nodal and extranodal lymphomas (Ryan *et al.*, 2008). However, the authors have attributed this to inferior treatment in the past. One of our patients died soon after the diagnosis was made and only 6 months from the appearance of superficial, small, palpable mass. Spread of an initially small tumour is also described in the literature (Anavekar *et al.*, 2007). Rapid course corresponds also to the high proliferation fraction. Thus, the prognosis of primary breast lymphoma should be realised as serious.

The disease course includes high risk of extranodal recurrence. High risk of progression within central nervous system similarly as in case of testicular lymphoma has been reported by some groups and denied by other researchers, in general ranging 5 – 10.2% (Ryan *et al.*, 2008; Yhim *et al.*, 2010). No extra-mammary spread was found in our patients.

The described prognostic factors include inclusion of anthracycline in the chemotherapy regimen and International Prognostic Index score (Ryan *et al.*, 2008). Radiation therapy is shown to reduce the risk of ipsilateral progression (Ryan *et al.*, 2008) although controversial views are published (Reyes *et al.*, 2005; Bonnet *et al.*, 2007). Rituximab has been shown effective in low risk B-cell lymphoma patients (Feugier *et al.*, 2005). However, the beneficial impact of rituximab on the survival and outcome was not significant in primary diffuse large cell lymphoma of the breast as well as in high-risk (according to age-adjusted International Prognostic Index score) patients in general (Feugier *et al.*, 2005; Neri *et al.*, 2008; Yhim *et al.*, 2010).

Surgical treatment generally seems to be not indicated for treatment of haematological diseases except palliative intervention or diagnostic purposes. Indeed, some authors have found that radical mastectomy is an adverse prognostic factor in case of breast lymphoma in comparison with lumpectomy (as in our patient) or biopsy (Jennings *et al.*, 2007; Ryan *et al.*, 2008). However, surgical treatment has been applied in 33.8% of patients in a recent series (Yhim *et al.*, 2010). Surgical intervention is also described as highly effective in selected cases unresponsive to chemoimmunotherapy including rituximab and to radiotherapy (Neri *et al.*, 2008).

At present, the described treatment recommendations include limited surgery or biopsy, followed by at least 3 cycles of anthracycline-based therapy and radiation therapy to the ipsilateral breast and regional nodes (Ryan *et al.*, 2008). However, first of all, the diagnosis should be established with certainty.

CONCLUSIONS

1. Breast lymphoma occurs in the local population with the incidence 0.63% of malignant breast tumours. The lesion retains significance for practising surgeon due to diagnostic pathway and occasional application of surgical treatment.
2. Diffuse large B-cell lymphoma is the most frequent type of breast lymphoma.
3. Immunohistochemical investigation is mandatory to identify breast lymphoma and perform reliable

differential diagnosis with other high-grade malignant tumours.

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Conflict of interest: None

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ORIGINAL ARTICLE

The Clinicopathologic Characteristics and Prognostic Significance of Triple – Negative Invasive Breast Cancer Phenotype

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Summary
Introduction. Triple-negative phenotype is defined by a primary tumor that is estrogen, progesterone and HER2/neu receptor negative. This cancer subtype is important because of its close relation to the basal-like breast cancer and its profound investigation could help to develop novel therapeutic strategies.
Aim of the study. Is to evaluate triple-negative breast cancer(TNBC) clinicopathological characteristics and prognostic significance.
Materials and Methods. We have retrospectively analyzed 76 unselected invasive breast cancer patients' cases diagnosed between 2005 and 2010 from RAKUS Pathological center and PSKUS. TNBC were defined as tumors that were estrogen, progesterone receptor negative, and HER2/neu negative. The clinicopathological features and prognostic significance were explored.
Results. 45 of 76 cases (59.2%) were defined as TNBC. The median follow-up from the original diagnosis until analysis was 24(range, 5- 48) months in the TNBC group and 22.6 (range, 3- 65) months in the non- TNBC group. The mean age at diagnosis was significantly younger for the TNBC group compared with non-TNBC group (55.5 versus 62.1years, respectively; $p < 0.021$). There was no statistically significant difference between the two groups in the T stage, lymph node status and histological type and stage of disease. Patients in the TNBC group were more likely to have grade III tumors (58.8% versus 29.2%; $p< 0.05$).
In the TNBC group in 2 (4.4%) patients' cases local recurrence occurred, versus 0 (0.0%) in the other group within the follow-up period ($p< 0.051$). The average time to local recurrence for patients with TNBC was 14 months (range, 12 - 16 months). A high proportion of patients with TNBC experienced distant recurrence compared with patients with non- TNBC (22.2% versus 0.0%, respectively; $p< 0.017$). The average time to distant recurrence for patients with TNBC was 14.2 months (range, 5 - 32 months). In the TNBC group 7 (15.5%) breast cancer-related deaths were detected versus no deaths in the other group, respectively.
Conclusion. TNBC was associated with younger age and higher histologic grade. TNBC had aggressive clinical course and high rate of early recurrence.
Key words: triple- negative, breast cancer.

INTRODUCTION

Breast cancer is the most common cancer among women in Latvia, accounting for approximately 900 of newly diagnosed cancers yearly. The breast cancer incidence rate in 2003 was 32.7 per 100.000 inhabitants per year, in 2004 – 34.0, but in 2005 32.2 cases. During last 10 years morbidity with breast cancer in Latvia increases per 2% yearly. The mortality rate in the last years slightly decreases and in 2005 death rate was 17.5 per 100,000 inhabitants (6, 7).

The systemic treatment of breast cancer has advanced toward a more targeted approach, which is aimed at specific molecular targets. Theoretically, this will minimize treatment risks and side effects and optimize benefits, particularly quality of life and overall survival. The goal is to perfect the approach so that each patient receives therapy targeted at her specific breast cancer subtype. Biomarkers, such as estrogen receptor (ER), progesterone receptor (PR), and HER2/neu are indicators of breast cancer prognosis and are considered in the selection of treatment. ER positivity predicts for response to endocrine therapy such as antiestrogen (tamoxifen) administration. HER2/neu positivity is useful for selecting targeted therapy with monoclonal antibody (trastuzumab) against HER2 (5, 8, 11, 12, 13, 14).

Recently, gene expression studies using DNA microarrays of invasive breast carcinoma has identified 5 distinct subtypes of morfologically similar breast cancers (luminal A, luminal B, normal breast-like subtype, ERBB2 (also known as HER2/neu) and basal-like (2, 10, 15).

The basal-like subtype neoplastic cells consistently express genes usually found in normal basal/ myoepithelial cells of the breast, including high-molecular-weight 'basal' cytokeratins (CK; CK5/6, CK14 and CK17), vimentin, p-cadherin, αB crystallin, fascin and caveolins 1 and 2. In population based studies, this subtype comprises approximately 15-20% of all breast cancers and is characterized by lack staining for estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2/neu), unresponsiveness to the usual endocrine therapies, shorter survival, and BRCA1- related breast cancer. The tumours often affect younger patients, show either p53 immunohistochemical expression or TP53 gene

mutations in up to 85% of cases, display exceedingly high levels of proliferation-related genes and express epidermal growth factor receptor (EGFR) in >60% of cases. Basal-like breast cancers are of high histologic grade, elevated mitotic count and have more aggressive clinical behaviour, a distinctive metastatic pattern and a poor prognosis despite responding to conventional neoadjuvant and adjuvant chemotherapy regimens (1, 2, 9, 16).

As basal-like breast cancers are ER, PR and HER2/neu negative, they are also called „triple- negative". But only in about 85% of triple-negative breast cancers basal-like subtype is confirmed when tested by appropriate immunohistochemical means (2).

As no satisfactory treatment is available for this cancer subtype we decided to analyse in this study the clinical characteristics and prognostic factors to help to develop adequate therapy strategies.

AIM OF THE STUDY

Is to evaluate triple-negative breast cancer clinicopathological characteristics and prognostic significance.

MATERIALS AND METHODS

76 patients with breast cancer, aged from 32 to 85 years, were retrospectively selected using specific immunohistochemical criteria from cases diagnosed in the period between 2005 and 2010 from RAKUS Pathology center and PSKUS.

For this study, triple-negative breast cancers were defined as those that were ER negative, PR negative, and HER2 negative. 45(59.2%) patients had TN breast cancer and 31(40.8%) had non- TN(Luminal- A, B) breast cancer.

Microscopic investigation

Histological parameters of all cases were reviewed by a pathologist. Histological type and grade of ductal breast cancers was determined for each case according to the Bloom-Richardson system. The specimens were selected based on the formalin-fixed, paraffin-embedded breast carcinoma tissue blocks from the primary tumors obtained from the archives of the RAKUS Pathology Center and PSKUS. In all 76 cases, the primary pathological diagnosis was confirmed on hematoxylin-eosin stained sections.

Immunohistochemical analysis

ER and PR status were determined using immunochemistry. For ER and PR, monoclonal antibodies were from *DakoCytomation*, *Glostrup*, Denmark, with cutoff levels for receptor positivity of more than 0%.

The assessment of HER-2/neu expression was carried out using the *HercepTest* kit according to the manufacturer's instructions.

Results interpretation

ER and PR are considered negative if immunoperoxidase staining of tumor cell nuclei is 0 %. HER2 was assessed through immunohistochemistry (IHC). IHC is scored on a qualitative scale from 0 to 3+, based on interpretation of staining intensity, with 0 and 1+ classified as negative (0- are considered, if staining of tumor cell membrane are less than 10%, and 1+, if more than 10% of tumor cell membrane stains partly)(Fig.1-3).

The outcomes were analysed in all 76 patients. Follow up has been maintained by reviewing clinical charts. The median follow-up from the original diagnosis until analysis was 24(range, 5- 48) months in the TNBC group and 22.6 (range, 3- 65) months in the non- TNBC group.

All patients have received surgical therapy and some of them have received chemotherapy and/or radiation therapy as well.

Relapses after 90 days were considered events. Local recurrence was considered as clinical and histological documented relapse in ipsilateral breast or regional lymphnodes.

Distant recurrence was considered as clinical disease distant evidence detected clinically and radiographically.

Relapse- free survival was defined as the time from diagnosis to development of first evidence of clinical or radiographic recurrence.

Statistical analysis: a SPSS statistical software version 12 and Microsoft Excell programm were used for statistical data analysis. Following data were analysed:

1) frequency analysis – were analysed frequencies,
2) descriptive statistics – were analysed minimal, maximal and average value, as well as standart deviation,
3) pair correlation analysis (Spearmans test), having detected p- value and correlation coefficient –r.

RESULTS

45 of 76 cases (59.2%) were defined as TNBC. The characteristics of the patients with TNBC and non-TNBC are compared in Table 1. The mean age at diagnosis was significantly younger for the TNBC group compared with non-TNBC group (55.5 versus 62.1years, respectively; $p < 0.021$). 16 (35.5%) of patients with TNBC were younger than 50 years compared with 5 (16.1%) of patients with non-TNBC ($p< 0.064$).

In the TNBC group: T1 were detected in 14 (41.1%) cases, T2- in 25 (55.5%), T3- in 2 (4.4%) cases, T4-in 4(8.9%) cases.

In the non- TNBC group: T1 were detected in 14 (45.1%) cases, T2- in 16 (51.6%), T3- in 1 (3.3%) case. There was no statistically significant difference between the two groups in T stage ($p< 0.108$).

The rate of node positivity was slightly higher in the non- TNBC group. In the TNBC in 19 (42.2%) cases were presented positive regional lymph nodes, in the other group in 16 (51.6%) cases lymph nodes were positive, but there was no statistically significant difference ($p<0.178$).

There was a correlation between tumor size and axillar lymph nodes ($p<0.001$) in both groups.

In 17 (25%) TNBC cases were diagnosed I stage, in 30 (44.1%) cases- II stage, in 19 (27.9%) cases- III stage and in 2 (3.0%) cases cancer were diagnosed IV stage. In the other group stage II was also the most frequent

cancer stage with 15 (48.4%) cases, followed by stage I with 11 (35.4%) cases detected and stage III with 5 (16.1%) cases detected. There was no statistically significant difference found between the two groups(p<0.030).

In the TNBC group in 34 (75.5%) cases there was ductal carcinoma detected, versus 24(77.4%) in the other group. In the TNBC group in 10 (22.2%) cases there was lobular carcinoma detected versus 5(16.1%) in other group. There was no statistically significant difference between the TNBC and other group in histological type (p< 0.638).

Patients in the TNBC group were more likely to have grade III tumors (58.8% versus 29.2%; p< 0.05).

In the TNBC group in 2 (4.4%) patients' cases local recurrence occurred. Both patients with local recurrences had previous modified radical mastectomy. No local recurrences occurred in the other group within the follow-up period (p< 0.051). The average time to local recurrence for patients with TNBC was 14 months (range, 12 - 16 months). A high proportion of patients with TNBC experienced distant recurrence compared with patients with non- TNBC (22.2% versus 0.0%, respectively; p< 0.017).

The average time to distant recurrence for patients with TNBC was 14.2 months (range, 5 - 32 months). In 6 (60%) cases distant metastases were diagnosed in one organ and in 4 (40%) cases in different organs simultaneously.

In 5 (50%) cases distant recurrence was detected in lungs, in 4 (40%) cases in bones, in 2 (20%) cases in liver, in 2 (20 %) cases in lymph nodes and in1 (10%) cases skin metastases were detected.

In the TNBC group 7 (15.5%) breast cancer-related deaths were detected versus no deaths in the other group, respectively. The median time to death for patients with TNBC was 22 months. The median survival time from recurrence to death was 11 months.

DISCUSSION

In the current study, TNBC was associated with younger age and high histologic grade.

There was no statistically significant difference between two groups in the T stage, stage of disease and histological type of the tumor.

In this study the rate of node positivity was slightly higher in the non- TNBC group, but this was no statistically significant difference. There was a correlation between tumor size and axillary lymph nodes (p< 0.001) in the TNBC group. This data is opposite to other studies' results there was no or weak correlation between T stage and metastases in lymph nodes (3). This contradicts the traditional opinion, that if tumor size increases the rate of node positivity increases as well. Foulkes at al. reported that this phenomen is also present in BRCA-associated cancers and suggested hematogenous spread of these cancers (4).

Our study addresses the short-term outcomes of patients with TNBC. According to the present study results the patterns of recurrence in the two groups were qualitatively different. Patients in the TNBC group experienced significantly higher rates of local and distant recurrences within the approximately first 2 years, in contrast to non-TNBC. This data reflects the aggressiveness of TNBC and correlates with Dent et al investigation results. They reported that TNBC had an increased likelihood of distant recurrence (hazard ratio, 2.6; 95% confidence interval, 2.0-3.5; P < 0.0001) and death (hazard ratio, 3.2; 95% confidence interval, 2.3-4.5; P < 0.001) within 5 years of diagnosis but not thereafter and that the risk of distant recurrence in the TNBC group peaked at first 3 years and declined rapidly after 4 years and no recurrences occurred after 8 years(3).

In our study local recurrence was detected in 4.4% of cases compared with 13% in other studies. This difference can be explained by different mean time to local recurrence (14 months compared with 2.8 years in other studies) and different median follow-up time (2 years compared with 8.1 years in other studies.) Thus large amount of recurrence is expected in later period (3).

We detected distant recurrence in 22.2% of cases compared with 33.9% in other studies. This difference can be explained by different mean time to distant recurrence (14.2 months compared with 2.6 years in other studies) and different median follow-up time (2 years compared with 8.1 years in other studies). Thus large amount of recurrence is expected in later period (3).

In the present study breast cancer- related deaths were detected in 7 (15.5%) cases versus no deaths in the other group. Unfortunately the short duration of follow-up made the overall survival analysis unfeasible.

CONCLUSION

TNBC was associated with younger age and higher histologic grade. TNBC had aggressive clinical course and high rate of early recurrence.

Conflict of interest: None

Table 1. Characteristics of TNBC versus non-TNBC

Variable	non-TNBC(N= 31), n (%)	TNBC(N= 45), n (%)
Mean age at diagnosis	62.1 years	55.1 years
Mean follow-up (m)	22.6m	24m
Lymph node status		
Positive	16(51.6%)	19(42.2%)
Negative	15(48.4%)	26(57.8%)
Tumor grade		
I	5(20.8%)	4(12.1%)
II	12(50%)	9(27.3.%)
III	7(29.2%)	20((60.6%)
ER levels	87.6%	0%
PR levels	67.4%	0%
HER2/neu negative	31(100%)	45(100%)

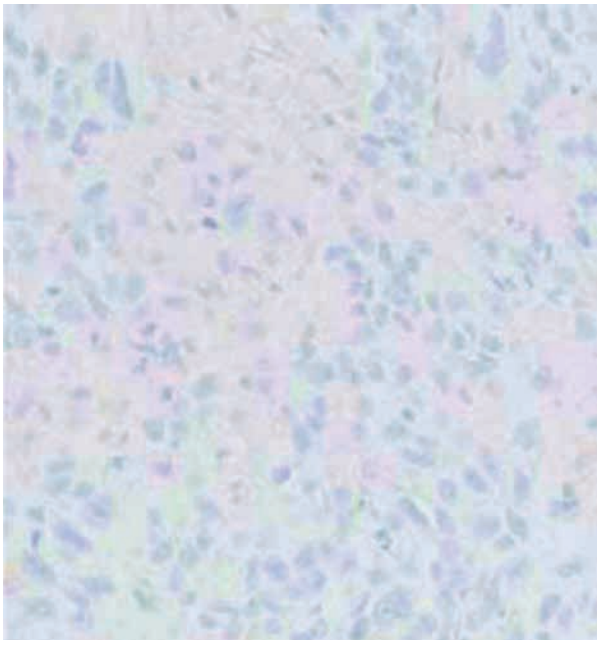

Fig. 1 ER receptor negative cancer. The monoclonal antibody DAKO-ER 1D5, x100

Fig. 2 PR receptor negative cancer. The monoclonal antibody DAKO- PgR 636, x100

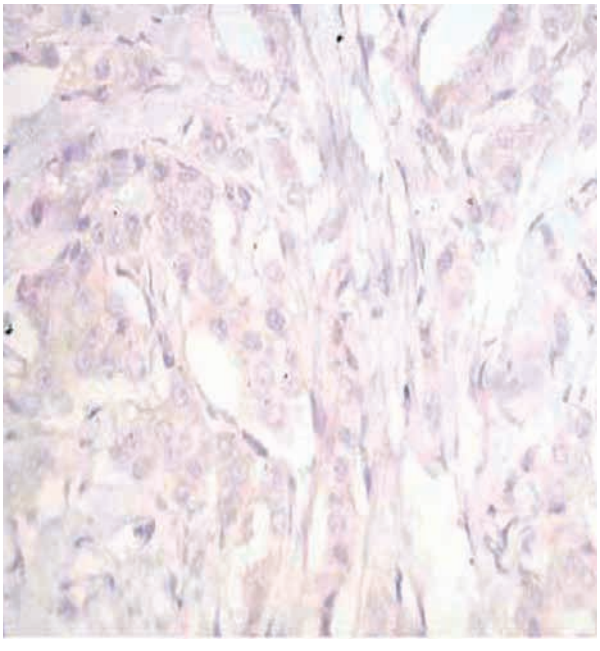

Fig. 3 Herceptest 0 result. Herceptest DAKO, x200

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ORIGINAL ARTICLE

Development of Breast-Conserving Surgery in Breast Cancer Treatment in Latvia (Last 20 Years Experience at Latvian Oncology Center)

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Summary
Introduction. Over the past 20 years, the incidence of breast cancer in Latvia increased more than twice as well as breast cancer surgery has increased by 127%. At the same time the structure of the operation types has changed, which is linked with the development of the modern method of treatment and surgical technique (oncoplastic surgery in particular). In 1990s breast conserving treatment (BCT) became an alternative to the mastectomy (ME) in breast cancer surgery in Latvia and frequencies of BCT increased more than five times from 1990 to 2010.
Aim of the study was to discover trends and changes in surgical treatment of breast cancer at Latvian Oncology Centre (LOC) over the past 20 years (1990-2010); to compare the long-term results of the BCT and the ME in general, considering the disease stage, the tumor site and the age of the patient.
Materials and methods. The results and data about 2413 female patients with breast cancer operated at LOC from January 1, 1990, to December 31, 2010, have been incorporated in the study. To track the trends, the data analysis has been carried out with a 5-year interval (from the 1st of January to the 31st of December in 1990th, 1995th, 2000th, 2005th and 2010th). Statistical computation was performed using the IBM SPSS Statistics version 19.0 for Mac OS X. Results were considered statistically significant when P value ≤ 0.05.
Results. Rate of BCT increased from 7% in 1990 to 33.6% in 2010, and the percentage of conservation of axilla rose from 1.5% in 2000 to 12% in 2010 ($p<0.001$). It is established that the survival rate varies greatly depending on the stage of disease and does not depend on the type of operation, tumor localization and the age of the patient. Analysis of the variation showed that long-term results do not statistically differ in both groups of patients after the BCT as well as the ME ($p<0.001$). Results of survival are reflected in the relevant tables.
Conclusions. 1. The study of the effect of 5-year and 10-year survival rate after the BCT and the ME acknowledges that treatment does not depend on the volume of operation (regardless of tumor site, stage of disease and age of the patient).
2. Breast cancer early detection and application of modern methods of operation, especially oncoplastic surgery, can significantly increase the proportion of BCT without compromising long-term results.
Key words: breast cancer, breast conserving treatment, oncoplastic surgery, axillary sentinel node biopsy, 5-year survival.

INTRODUCTION

Breast cancer (BC) is mainly prevalent among women (approximately 25% of all tumors) and there are more than one million new cases all over the world annually (10). In Europe breast cancer is the most frequent cause of death of all cancers for women and the most common cause of death for women aged 35 to 65 (22).

In Latvia, there are nearly 1,000 new breast cancer cases per year and one in ten Latvian women is under the risk to get breast cancer in her lifetime (4, 14).

For the period of the last 30 years the incidence of breast cancer in Latvia has doubled (from 461 new breast cancers in 1978 to 1056 in 2008) and mortality rate in breast cancer cases in absolute terms increased by 103.4% (from 231 fatalities in 1978 to 470 in 2008). (4, 14, 23, 24)

Breast cancer is less common at a young age, but the risk of breast cancer is dramatically increasing for women after the age of 40. In Latvia in 2009, 938 patients (94.5% of all breast cancers) were women above 40, and the dominant age group was 50-69 years old patients – 499 women (50.5%). In 40-49 years group there were 166 women (17%), and in 70 years group – 273 women (27%). (24)

Unfortunately, in Latvia a considerable part of patients seek for help when it is too late, consequently about one-third of women at the time of diagnosis appeared to have III-IV stage. In 2008 at the moment of diagnosis stage I was revealed among 26% of patients, stage II – among 36,2%, stage III- among 23,4%, and stage IV – among 7,9% (23, 24).

Delayed diagnosis of breast cancer, and accordingly the delay of treatment has a significant impact on survival rate – it has not changed radically over the last 30 years in Latvia. The 5-year survival rate in breast cancer patients in Latvia in 2008 was 66,2% (23).

All breast cancer patients in Latvia are operated at four of the existing local medical institutions – Latvian Oncology Center (LOC) of Riga Eastern University Hospital, Stradins Clinical University Hospital, Oncological Clinic of Daugava's Hospital (Daugavpils), Oncological Clinic of Seaside Hospital (Liepaja). The highest number of breast cancer surgery is performed at Latvian Oncology Centre (in 2008 it was 607 patients out of 898, or 67,6%) (24).

Surgical treatment of breast cancer has a long history; however, revolutionary changes have occurred in the late 19th century when Halsted developed a technique of radical mastectomy. As a result of these operations, the 20-year survival rate for breast cancer patients increased from 10 to 50%. (17) In the second half of the 20th century dramatic changes in the surgical treatment of breast cancer took place: in 1970s as an alternative to Halsted radical mastectomy, more moderate, but no less effective Madden modified mastectomy was introduced. Furthermore, in another 10-15 years, as an alternative to mastectomy, breast conserving surgery with radiotherapy were pioneered (2, 5, 6, 19, 20). Since 1995 sentinel lymph node biopsy has become widely used and the introduction of oncoplastic surgery has brought in new possibilities for breast conservation in the cases where mastectomy has been previously performed (1, 3, 7, 9, 11, 12, 13).

Nowadays, the basic principles of surgical treatment of breast cancer are:

1. Breast conserving treatment (BCT) using oncoplastic surgery instead of mastectomy.
2. Sentinel lymph node biopsy preserving axillary lymph nodes when possible.
3. Immediate or delayed breast reconstruction if the breast cannot be preserved.

In recent decades surgical treatment of breast cancer in Latvia in compliance with the latest trends in the world has also changed. With the introduction of oncoplastic surgery the proportion of breast conservation surgery has notably increased (over the last 20 years it has increased 5 times), besides, the 5-year survival results of the BCT have improved as well (4, 14). Sentinel node biopsy and breast reconstruction in Latvia are used more frequently now. However, this raise has not reached the world's level yet, if we talk about the quantity of primary breast reconstructions, for instance. (8, 15, 16, 18, 21)

AIM OF THE STUDY

The study aims to analyze breast cancer surgical treatment trends in Latvia for the period of the last 20 years, the development of modern treatment methods, especially, oncoplastic surgery, the influence of the tactics of surgical treatment, and the effects of these changes on the structure of operation (ratio of breast conserving operations and mastectomies) and long-term results of treatment.

The survival of patients with breast cancer after BCT in Latvia has not been studied yet.

Hypothesis: BCT (including oncoplastic operation) frequency increase does not impair the results of BC treatment.

MATERIALS AND METHODS

The retrospective study uses the data of the Department of Statistics of Riga Eastern University Hospital and the Latvian Cancer Registry of Latvian Oncology Centre (since July 1, 2009, – The Register of Patients Suffering from Particular Diseases of The Centre of Health Economics), as well as data from the Latvian Oncology Centre operation logs and patient's medical records (outpatient medical card (Form No.025/s), inpatient medical card (Form No.003/s)).

Female patients operated at the Latvian Oncology Center from the 1st of January 1990 to the 31st of December 2010 have been included in the study. Merely primary surgeries have been examined; re-operations (recurrence, metastasis treatment and other operations) have not been included in the study. In the case of bilateral breast cancers (both synchronous and metachronous) each incidence has been considered separately as the independent condition (new case).

To track the trends, the data analysis has been carried out with a 5-year interval (from 1st of January to 31st of December of 1990th, 1995th, 2000th, 2005th and 2010th). Overall, 2413 patients' data has been investigated.

The total number of operations in each of the analyzed years and the types of operation have been examined (all operations have been divided into two groups: operations which result in complete breast removal (mastectomy - ME) and surgery when part of the breast was preserved (breast conserving treatment - BCT)); the structure of the operation has been analyzed as well, considering the tumor localization, stage and patient age.

Statistical computation was performed using the IBM SPSS Statistics version 19.0 for Mac OS X (SPSS Inc., USA). Results were considered statistically significant when P value ≤ 0.05. Categorical data was compared using the Chi-Square test of Contingency, hypotheses were evaluated with One-Way Analysis of Variance (ANOVA) method.

Using the medical statistic's methods the changes in the structure of surgeries for patients operated from 1990th to 2010 have been compared and analyzed, considering the breast cancer stage and patient age. The 5 and 10 year survival results for patients operated in 1990th, 1995 and 2000 have been analyzed considering the breast cancer stage, type of surgery and tumor localization. Though, for the patients operated in 2005 the 5-year survival results have been studied.

RESULTS

Totally more than 10,000 breast cancer female patients have been operated at Latvian Oncology Center from January 1990 to December 2010. To keep the track of surgical treatment trends, data analysis has been performed with a 5-year interval (1990th, 1995th, 2000th, 2005th and 2010). Overall, the data for 2413 operations has been processed.

During the period from 1990 to 2010 some serious changes in the structure of operations have happened. The proportion of BCT has increased five times (from

7% in 1990 to 33.6% in 2010) (p<0.001), with the main raise in the year 2000 (increase from 7% in 1990 to 26.1% in 2000) (p<0.001) along with the increase of the percentage of BCT in 2005 (7%) and another slight boost increase by the year 2010 (20%) (Table 1).

Table 1. Number of breast cancer operations at Latvian Oncology Center in 1990, 1995, 2000, 2005, 2010

Operation		Year					Total
		1990	1995	2000	2005	2010	
Mastectomia	No. of op.	238	353	441	419	366	1817
	%	93.0	82.7	73.9	72.0	66.4	75.3
Breast conserving operations	No. of op.	18	74	156	163	185	596
	%	7.0	17.3	26.1	28.0	33.6	24.7
Total	No. of op.	256	427	597	582	551	2413
	%	100.0	100.0	100.0	100.0	100.0	100.0

Having analyzed breast cancer surgery according to the distribution of breast cancer stages, it appeared that the distribution of stage-operated patients over the period has significantly changed – more and more breast operations have been carried out in the early stages of breast cancer. In 1990 among the operated patients I-II stage group figured to 64.1%, however in 1995 the percentage of the early stage rose to 78.5% and stabilized at this level during the following years (81.9% in 2000, 80.8% in 2005). (Table 2).

Table 2. All breast cancer operations by stage performed at Latvian Oncology Center in 1990, 1995, 2000, 2005

Stage		Year				Total
		1990	1995	2000	2005	
0	No. of patients	3	0	4	16	23
	%	1.2	0.0	0.7	2.7	1.2
I	No. of patients	50	113	174	190	527
	%	19.5	26.5	29.1	32.6	28.3
II	No. of patients	111	222	311	265	909
	%	43.4	52.0	52.1	45.5	48.8
III	No. of patients	82	81	89	101	353
	%	32.0	19.0	14.9	17.4	19.0
IV	No. of patients	10	11	19	10	50
	%	3.9	2.6	3.2	1.7	2.7
Total	No. of patients	256	427	597	582	1862
	%	100.0	100.0	100.0	100.0	100.0

Furthermore, the exploration of the patients by the age group in each study year has been carried out. As a result, the tendency of the decrease of the proportion of younger patients (<40 years of age) and the increase of the proportion of patients over 70 years of age has been revealed. However, the patients in the age groups from 40 to 69 remain on the same level (median is 60 years, mean 59.36, range from 24 to 94 years) (Table 3).

Table 3. Number and percentage of breast cancer female patients by age with breast cancer operations performed at Latvian Oncology Center in 1990, 1995, 2000, 2005, 2010

Age groups of breast cancer female patients		Year					Total
		1990	1995	2000	2005	2010	
<40 years	No. of patients	25	44	32	30	25	156
	%	9.8	10.7	5.5	5.3	4.7	6.6
40-49 years old	No. of patients	55	77	111	113	82	438
	%	21.7	18.7	19.0	20.0	15.3	18.6
50-59 years old	No. of patients	60	108	140	135	132	575
	%	23.6	26.2	24.0	23.9	24.6	24.5
60-69 years old	No. of patients	76	107	169	159	161	672
	%	29.9	26.0	29.0	28.1	30.0	28.6
>70 years	No. of patients	38	76	131	128	136	509
	%	15.0	18.4	22.5	22.7	25.4	21.7
Total	No. of patients	254	412	583	565	536	2350
	%	100.0	100.0	100.0	100.0	100.0	100.0

The study of the 1862 patients according to tumor localization has been performed. This analysis has been made using the SEER site-specific code (SSC) C50.0-C50.9. Diverse information over the years has been acquired along with the facts of highly significant fluctuations over the years at one location (Table 4).

Table 4. Breast cancer female patient distribution by breast cancer (C50) subsites (C50.0–C50.9*) at Latvian Oncology Center in 1990, 1995, 2000, 2005

Breast cancer (C50) subsites (by site-specific code (SSC))		Year				Total
		1990	1995	2000	2005	
C50.0	No. of patients	49	91	16	8	164
	% within C50	19.1	21.3	2.7	1.4	8.8
C50.1	No. of patients	20	20	8	4	52
	% within C50	7.8	4.7	1.3	0.7	2.8
C50.2	No. of patients	18	34	44	11	107
	% within C50	7.0	8.0	7.4	1.9	5.7
C50.3	No. of patients	8	8	14	6	36
	% within C50	3.1	1.9	2.3	1.0	1.9
C50.4	No. of patients	75	138	180	49	442
	% within C50	29.3	32.3	30.2	8.4	23.7
C50.5	No. of patients	28	18	20	5	71
	% within C50	10.9	4.2	3.4	0.9	3.8
C50.6	No. of patients	0	0	0	1	1
	% within C50	0.0	0.0	0.0	0.2	0.1
C50.8	No. of patients	9	67	100	36	212
	% within C50	3.5	15.7	16.8	6.2	11.4
C50.9	No. of patients	49	51	215	462	777
	% within C50	19.1	11.9	36.0	79.4	41.7
Total	No. of patients	256	427	597	582	1862
	% within C50	100.0	100.0	100.0	100.0	100.0

* Site Codes: C50.0 - Nipple, C50.1 - Central portion of breast, C50.2 - Upper-inner quadrant of breast, C50.3 - Lower-inner quadrant of breast, C50.4 - Upper-outer quadrant of breast, C50.5 - Lower-outer quadrant of breast, C50.6 - Axillary tail of breast, C50.8 - Overlapping lesion of breast, C50.9 - Breast unspecified, multi-focal neoplasm in more than one quadrant of the breast.

Having researched the types of operations depending on the tumor over the years, it has been found that in 1990 and 1995 breast conserving operations were carried out more frequently if the tumor localized in the upper outer quadrant (C50.4), in 2000 – in upper outer quadrant (C50.4) and when the subsite was unspecified (C50.9), but in 2005 – only when the subsite was unspecified (C50.9) (Table 5).

Table 5. Type of breast cancer surgical operations – breast conserving treatment (BCT) – by breast cancer (C50) subsites (C50.0–C50.9*) at Latvian Oncology Center in 1990, 1995, 2000, 2005

Breast cancer (C50) subsites	Year												Total
	1990			1995			2000			2005			
	total no.	BCT		total no.	BCT		total no.	BCT		total no.	BCT		
		n	%		n	%		n	%		n	%	
C50.0	49	2	4.1	91	17	18.7	16	2	12.5	8	3	37.5	164
C50.1	20	2	10.0	20	4	20.0	8	2	25.0	4	0	0	52
C50.2	18	1	5.6	34	4	11.8	44	14	31.8	11	0	0	107
C50.3	8	1	12.5	8	1	12.5	14	3	21.4	6	2	33.3	36
C50.4	75	6	8.0	138	31	22.5	180	54	30.0	49	17	34.7	442
C50.5	28	2	7.1	18	2	11.1	20	9	45.0	5	2	40.0	71
C50.8	9	1	11.1	67	6	9.0	100	13	13.0	36	11	30.6	212
C50.9	49	3	6.1	51	9	17.6	215	59	27.4	463	128	27.7	778**
Total	256	18	7.0	427	74	17.3	597	156	26.1	582	163	28.0	1862

* Site Codes: C50.0 - Nipple, C50.1 - Central portion of breast, C50.2 - Upper-inner quadrant of breast, C50.3 - Lower-inner quadrant of breast, C50.4 - Upper-outer quadrant of breast, C50.5 - Lower-outer quadrant of breast, C50.6 - Axillary tail of breast, C50.8 - Overlapping lesion of breast, C50.9 - Breast unspecified, multi-focal neoplasm in more than one quadrant of the breast.

** C50.6 (n=1) added.

The 5-year and 10-year survival depending on the breast cancer subsite (C50.0–C50.9) has been studied. The analysis has not revealed any credible statistical difference ($p<0.001$) in 5-year and 10-year survival between different breast cancer subsites (Table 6).

Table 6. 5-year and 10-year survival in breast cancer female patients operated at Latvian Oncology Center in 1990, 1995, 2000, 2005* by breast cancer (C50) subsites (C50.0–C50.9)**

Breast cancer (C50) subsites	5-year survival				10-year survival			
	Total no. of patients	% within C50	Survive ≥5 years		Total no. of patients	% within C50	Survive ≥10 years	
			No. of patients	%			No. of patients	%
C50.0	164	8.8	107	65.2	156	12.2	75	48.1
C50.1	52	2.8	38	73.1	48	3.8	27	56.3
C50.2	107	5.8	81	75.7	96	7.5	53	55.2
C50.3	36	1.9	25	69.4	30	2.3	18	60.0
C50.4	441	23.7	329	74.6	393	30.7	221	56.2
C50.5	71	3.8	54	76.1	66	5.2	38	57.6
C50.8	211	11.4	113	53.6	176	13.8	64	36.4
C50.9	776	41.7	559	72.1	315	24.6	191	60.6
Total	1858	100.0	1306	70.3	1280	100.0	687	53.7

***** 5-year survival only.

** Site Codes: C50.0 - Nipple, C50.1 - Central portion of breast, C50.2 - Upper-inner quadrant of breast, C50.3 -

Lower-inner quadrant of breast, C50.4 - Upper-outer quadrant of breast, C50.5 - Lower-outer quadrant of breast, C50.6 - Axillary tail of breast, C50.8 - Overlapping lesion of breast, C50.9 - Breast unspecified, multi-focal neoplasm in more than one quadrant of the breast.

Having explored breast conserving operations performed at the Latvian Oncology Center depending on cancer stage, it can be observed that the BCT is most often performed in stage I breast cancer patients. BCT share is increasing significantly over years (Table 1). However, this trend is particularly evident with the stage I breast cancer female patients (from 14% in 1990 to 47.4% in 2005) ($p<0.05$) (Table 7).

Table 7. Type of breast cancer surgical treatment – mastectomy (ME) and breast conserving treatment (BCT) – by breast cancer stage at Latvian Oncology Center in 1990, 1995, 2000, 2005

Stage		Year											
		1990			1995			2000			2005		
		Operations			Operations			Operations			Operations		
		ME	BCT	Total	ME	BCT	Total	ME	BCT	Total	ME	BCT	Total
0	n	2	1	3	0	0	0	4	0	4	10	6	16
	%	66.7	33.3	100.0	0.0	0.0	0.0	100.0	0.0	100.0	62.5	37.5	100.0
I	n	43	7	50	79	34	113	96	78	174	100	90	190
	%	86.0	14.0	100.0	69.9	30.1	100.0	55.2	44.8	100.0	52.6	47.4	100.0
II	n	103	8	111	187	35	222	240	71	311	208	57	265
	%	92.8	7.2	100.0	84.2	15.8	100.0	77.2	22.8	100.0	78.4	21.6	100.0
III	n	80	2	82	76	5	81	83	6	89	91	10	101
	%	97.6	2.4	100.0	93.8	6.2	100.0	93.3	6.7	100.0	90.1	9.9	100.0
IV	n	10	0	10	11	0	11	18	1	19	10	0	10
	%	100.0	0.0	100.0	100.0	0.0	100.0	94.7	5.3	100.0	100.0	.0	100.0
Total	n	238	18	256	353	74	427	441	156	597	419	163	582
	%	93.0	7.0	100.0	82.7	17.3	100.0	73.9	26.1	100.0	72.0	28.0	100.0

Having evaluated the 5-year and 10-year survival results according to breast cancer stage, the strong dependence on the stage at which the disease is diagnosed has proven to be found – survival is considerably better in patients with breast cancer at stage I and II in comparison with III-IV stage patients. This tendency can be revealed by examining breast cancer surgical treatment outcomes after BCT and even after a mastectomy (Table 8).

Table 8. 5-year and 10-year survival by breast cancer stage and type of operation (ME or BCT) in breast cancer female patients operated at Latvian Oncology Center in 1990, 1995, 2000, 2005*

Stage	Operations	Years 1990, 1995, 2000, 2005		Years 1990, 1995, 2000	
		operated petients	5-year survival	operated petients	10-year survival
0	ME	16	14	6	4
		100.0%	87.5%	100.0%	66.7%
	BCT	7	7	1	1
		100.0%	100.0%	100.0%	100.0%
	Total	23	21	7	5
		100.0%	91.3%	100.0%	71.4%
I	ME	318	268	218	160
		100.0%	84.3%	100.0%	73.4%
	BCT	209	183	119	90
		100.0%	87.6%	100.0%	75.6%
	Total	527	451	337	250
		100.0%	85.6%	100.0%	74.2%
II	ME	736	536	530	286
		100.0%	72.8%	100.0%	54.0%
	BCT	170	128	114	61
		100.0%	75.3%	100.0%	53.5%
	Total	906	664	644	347
		100.0%	73.3%	100.0%	53.9%
III	ME	329	151	239	77
		100.0%	45.9%	100.0%	32.2%
	BCT	23	11	13	3
		100.0%	47.8%	100.0%	23.1%
	Total	352	162	252	80
		100.0%	46.0%	100.0%	31.7%
IV	ME	49	8	39	5
		100.0%	16.3%	100.0%	12.8%
	BCT	1	0	1	0
		100.0%	.0%	100.0%	.0%
	Total	50	8	40	5
		100.0%	16.0%	100.0%	12.5%
Total	ME	1448	977	1032	532
		100.0%	67.5%	100.0%	51.6%
	BCT	410	329	248	155
		100.0%	80.2%	100.0%	62.5%
	Total	1858	1306	1280	687
		100.0%	70.3%	100.0%	53.7%

* 5-year survival only.

These illustrate that the survival after BCT was a little better (has been found no statistical difference) than that after mastectomy in patients with breast cancer at I-II stages (Table 8).

DISCUSSION

Over the past 20 years the incidence of breast cancer in Latvia increased more than twice as well as breast cancer surgery has increased by 127%. At the same time the structure of the operation types has changed which is closely linked with the development of the modern methods of treatment (4, 14, 23, 24). Having analyzed the results, it becomes evident that breast cancer treatment in Latvia has changed in relation to the world trends. The total number of breast cancer operations increased rapidly over 10 years (from 1990 to 2000) from 256 to 597, these results can be explained by improving diagnostics, but over the past 10 years the number of operations hasn't changed radically (582 pts in 2005, 551 pts in the year 2010).

In 1990s BCT became an alternative to mastectomy in breast cancer treatment in Latvia and the incidence of BCT increased from 7.0% in 1990 to 33.6% in 2010. This raise was influenced by several factors which included apart from the development of the surgical technique (especially oncoplastic surgery) and changes of tactics of treatment, the diagnostic improvement, as evidenced by the expansion of the early stages in patients' population.

The five times proportion increase of the BCT among all breast cancer surgeries performed at Latvian Oncology Center for the period of 1990 until 2010 can be divided into four stages:

- the first BCT share increase from 7% to 17.3% (for 143%) for the period of 5 years (from 1990 to 1995) of the Latvian Oncology Center is related to the implementation of the breast conserving surgery with radiation instead of mastectomy;
- in the next 10 years at the Latvian Oncology Center the BCT share has increased by 64.7% (during the first 5 years increased by 41.2%; however, since 2000 until 2005 this proportion rose only by 14, 7% (respectively, 26.1% and 28%), presumably because of the traditional BCT options had been through;
- in the second half of 2000s new opportunities for breast conservation have emerged after the development of oncoplastic surgery in Latvia. Over the past 5 years, the major progress of the BCT share increase of 28.7% – from 28% in 2005 to 33.6% in 2010 can be observed.

Having questioned whether there is a correlation between tumor localization and type of surgery, as well as whether survival depends on tumor localization, the relevance of this link has not been found. However, it should be noted that the evaluation of breast tumor localization based on the site-specific code entries might be false since very different results for the localization of the structure in different years have been obtained.

Having analyzed the survival results according to the stage of breast cancer, the strong relation to the disease diagnosis stage can be seen – survival is significantly better in patients with breast cancer stages I and II than III-IV.

Having examined the structure of the operation, depending on the stage of the process, it has been revealed that the BCT surgery has still been seldom applied to stage II and occasionally to stage III breast cancer. At stages I and II the 5 and 10 year survival after BCT and ME does not differ statistically. There has been found no statistical difference in the 5-year survival rate among stage III patients.

CONCLUSIONS

1. When analyzing the 5-year and 10-year survival results according to the methods used in the surgical treatment of breast cancer, it can be concluded that with the same stages of breast cancer patients' survival is the same as after the mastectomy and the BCT.
2. Breast cancer early detection and application of modern methods of operation, especially, oncoplastic surgery, can significantly increase the proportion of BCT not diminishing long-term results of treatment.

Conflict of interest: None

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ORIGINAL ARTICLE

Histological and Immunohistochemical Evaluation of Cytokeratin 5/6 Expressing Breast Cancer

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Summary
Introduction. Breast cancer represents morphologically, molecularly and prognostically heterogeneous group of tumours. In order to characterise the prognosis more exactly, molecular subtyping has been developed. However, the role of basal markers, including cytokeratin 5/6, (CK5/6) in the immunohistochemical typing of breast cancer still causes significant controversy.
Aim of the study was to determine the frequency of CK5/6 expression in invasive breast cancers in Latvian patients and to characterize CK5/6 positive tumours by histology and molecular subtypes in order to clarify the diagnostic role of CK5/6 expression in breast cancer.
Materials and methods: Consecutive potentially radically operated invasive breast cancer cases were identified by archive search. The gross and microscopic evaluation was performed on breast cancer protocol basis, aiming at complete description of morphological prognostic factors. Expression of oestrogen (ER) and progesterone (PR) receptors, actin, p53, p63, CK5/6 and Ki-67 was performed by immunohistochemistry. HER2 protein over-expression was detected by HercepTestTM. Molecular subtypes (luminal A, luminal B, HER-2 positive or triple negative) were determined for each tumour using ER, PR, HER-2 expression data. Descriptive statistics including calculation of 95% confidence interval (CI) was carried out by CIA software.
Results: Positive CK5/6 expression in tumour cells was observed in 23 (15.9%) cases [95% CI = 10.8-22.7]. No statistically significant differences were found between CK 5/6 positive cases and CK 5/6 negative controls regarding the histological type and grade of breast cancer as well as age of cancer diagnostics. CK 5/6 positive cases mostly were of luminal A (47.8%; 95% CI = 29.2-67.0) or triple negative (43.5%; 95% CI = 25.6-63.3) molecular subtype. CK5/6 expression was found in 43.4 % [95% CI = 25.6-63.1] of triple negative breast cancer cases in contrast to the expression rate 10.7% [95% CI = 6.3-17.3] in other molecular subtypes.
Conclusions: Frequency of CK5/6 expression is sufficient to carry out the diagnostic examination. CK 5/6 expression is not limited to single molecular type. Luminal A and triple negative breast cancer constitute the largest groups within CK5/6 positive cases. CK5/6 expression is significantly more frequent in triple negative breast cancer than in other molecular types. However, CK5/6 as a complementary marker for triple negative breast cancer is characterised by low sensitivity although high specifity. CK5/6 positive tumours tend to have higher proliferation fraction.
Key words: breast cancer, molecular subtypes, cytokeratin 5/6.

INTRODUCTION

Breast cancer represents morphologically, molecularly and prognostically heterogeneous group of tumours. In order to describe the prognosis of individual patient more exactly, the histological classification of breast cancer by World Health Organization could be combined by DNS-microarray analysis in order to determine the molecular subtypes. However, gene expression microarrays have not become a routine practice in pathology laboratories yet. In contrast, immunohistochemistry (IHC) nowadays is a routine investigation. IHC markers like expression of oestrogen (ER) and progesterone (PR) receptors, and HER-2 protein can be used as surrogates for DNA-microarrays in subtyping the breast cancer (17, 23). According to these main factors initially breast cancer was classified in four molecular subtypes (13) including luminal A (ER+, PR+, HER-2-), luminal B (ER+, PR+, HER-2+), HER-2 positive (ER-, PR-, HER-2+) and triple negative (ER-, PR-, HER-2-). Considering other IHC markers (Cytokeratin 5/6 (CK5/6), p53 family member p63, epidermal growth factor receptor, P-cadherin), some subtypes in this molecular classification can be subdivided and supplemented (16).

By IHC, *Nielsen et al.* divided breast cancer in three subtypes: luminal (ER+, HER-2-), HER-2 (HER-2+) and basal-like (ER-, HER-2-, CK5/6+ or HER-1+) (12). *Carey et al.* updated IHC subtype definition as luminal A (ER+ and/or PR+, HER-2-), luminal B (ER+ and/or PR+, HER-2+), HER-2+/ER- (ER-, PR-, HER-2+), basal-like (ER-, PR-, HER-2-, CK5/6+) and unclassified (negative for all markers) (4). However, there is still no internationally accepted breast cancer molecular subtype classification, and the validity of additional markers like CK 5/6 is a matter of debates (17).

THE AIM OF THE STUDY

In order to clarify the diagnostic role of CK5/6 expression in breast cancer, the aim of the present study was to determine the frequency of CK 5/6 expression in invasive breast cancers in Latvian patients and to characterize CK5/6 positive tumours by histology and molecular subtypes.

MATERIALS AND METHODS

The study group comprised 145 consecutive potentially radically operated invasive breast cancer cases, identified by archive search. The gross and microscopic evaluation was performed on breast cancer protocol basis, aiming at complete description of morphological prognostic factors. The tissues were fixed in neutral buffered formalin, processed in vacuum infiltration processor Tissue-Tek® VIP™ 5 (Sakura Seiki Co.,Ltd., Nagano, Japan) and embedded in paraplast (Diapath S.r.l., Belgamo, Italy) using tissue embedding system TES 99 (Medite GmbH, Burgdorf, Germany). Four-micron-thick sections routinely were stained with haematoxylin-eosin. The formalin-fixed, paraffin-embedded tissues, cut at 3 micron thick sections on electrostatic slides (Histobond, Marienfeld, Germany) were investigated by IHC, using heat-induced epitope retrieval in TEG buffer at pH 9.0 in microwave oven 3x5 min. Panel of primary antibodies against oestrogen receptor alpha (clone 1D5, dilution 1:1), progesterone receptors (clone PgR636, 1:1), actin (clone HHF35, 1:400), p53 (clone DO-7, 1:400), p63 (clone 4A4, 1:200), CK5/6 (clone D5/16B4, 1:100) and Ki-67 (clone MIB-1, 1:100) was employed. Peroxidase-conjugated labelled streptavidin-biotin visualization system was applied for the detection of bound primary antibodies, followed by colour development by 3,3'-diaminobenzidine. All IHC reagents were produced by Dako, Glostrup, Denmark. HER2 protein over-expression was detected by HercepTest™ according to manufacturer's (Dako, Glostrup, Denmark) instructions. Appropriate positive and negative controls were performed. Breast cancers expressing ER and PR in less than 10% of neoplastic cells were considered negative for hormone receptor expression (15). The tumour was included in CK5/6 positive group, if the cell cytoplasm showed diffuse, strong CK5/6 expression. Molecular subtypes (luminal A, luminal B, HER-2 positive or triple negative) were determined for each tumour using ER, PR, HER-2 expression data (13). Descriptive statistics including calculation of 95% confidence interval (CI) was carried out by CIA software (1).

RESULTS

In the described period, 158 women underwent potentially radical operation for invasive breast cancer. By IHC, CK5/6 expression was evaluated in 145 (91.8%) cases. Positive CK5/6 expression in tumour cells (Figure 1) was observed in 23 (15.9%) cases [95% CI = 10.8-22.7]. The mean age of patients was 59.7 years [54.5-65] ranging 34-80 years in CK5/6 positive group, and 59.9 years [57.8-62] ranging 35-86 years in CK5/6 negative group. The CK5/6 positive group was represented mainly by invasive ductal breast cancer – 69.6% [49.1-84.4], followed by lobular and medullary cancer. The complete profile of CK5/6 positive breast cancer is represented in Table 1. Among CK 5/6 positive ductal breast cancers, high grade (G3) cases (Figure 2) were the most frequent group (Table 1). In CK5/6 negative group, invasive ductal breast cancer constituted 78.0% [70.0-

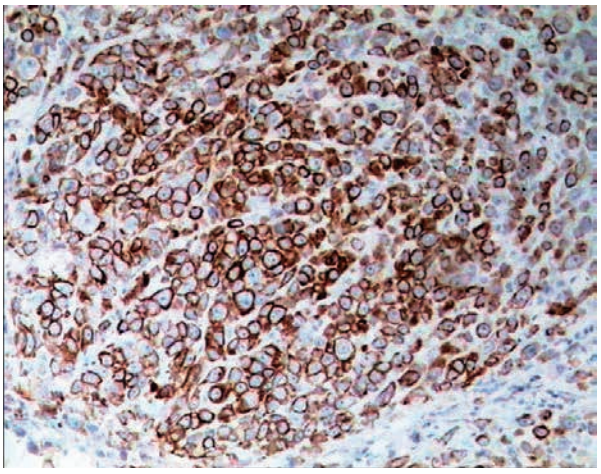

Fig. 1. Cytokeratin 5/6 expression in the cytoplasm of neoplastic cells. Immunoperoxidase. Original magnification x100.

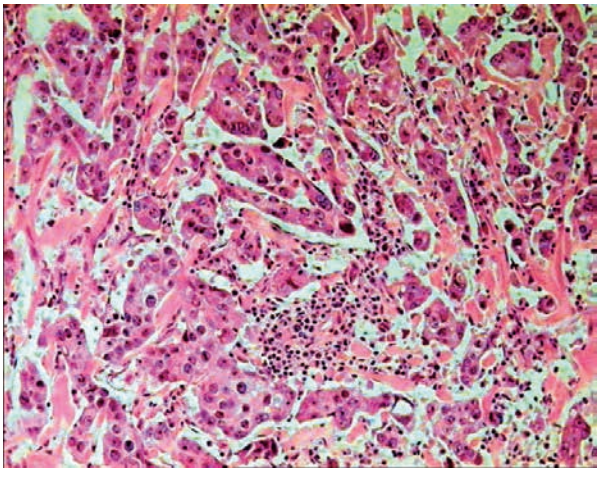

Fig. 2. High grade invasive breast cancer. Haematoxylin-eosin. Original magnification x100.

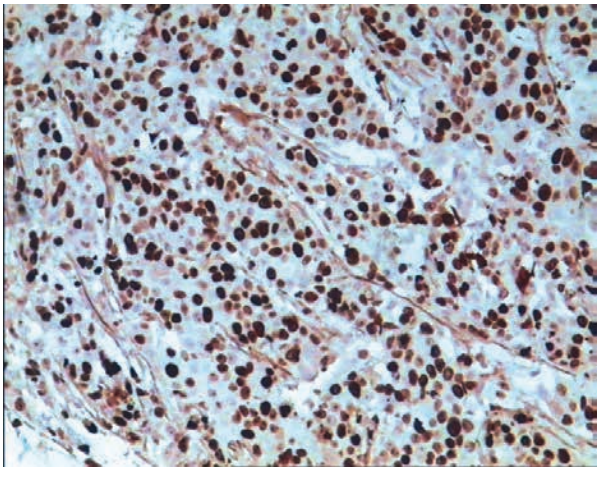

Fig. 3. Proliferation fraction by Ki-67 in the invasive breast cancer. Immunoperoxidase. Original magnification x100.

Table 1. Pathological characteristics of CK5/6 positive and negative breast cancer

	CK5/6 positive group		CK5/6 negative group	
	Frequency (%)	95% Confidence interval	Frequency (%)	95% Confidence interval
Histologic subtype				
Invasive ductal breast cancer	69.6	49.1-84.4	78	70.0-84.3
Invasive ductal breast cancer with mucinous component	0	0-14.3	1.6	0.4-5.8
Invasive ductal breast cancer with papillary component	0	0-14.3	0.8	0.1-4.5
Invasive lobular cancer	17.4	7.0-37.1	14.8	9.5-22.1
Typical medullary cancer	8.7	2.4-26.8	0	0-3.0
Atypical medullary breast cancer	0	0-14.3	0.8	0.1-4.5
Invasive cribriform carcinoma	0	0-14.3	1.6	0.4-5.8
Apocrine breast cancer	0	0-14.3	0.8	0.1-4.5
Metaplastic breast cancer	0	0-14.3	0.8	0.1-4.5
Mucinous breast cancer	0	0-14.3	0.8	0.1-4.5
Undifferentiated breast cancer	4.3	0.7-21.0	0	0-3.0
Grade of invasive ductal breast cancer				
G1	6.3	1.1-28.3	8.4	4.3-15.7
G2	12.5	3.5-36.0	40	30.7-50.0
G3	81.2	57.0-93.4	51.6	41.7-61.3
Expression of other immunohistochemical markers				
p53	47.8	29.2-67.0	24.6	17.8-32.9
p63	8.7	2.4-26.8	3.2	1.2-8.1
Actin	0	0-14.3	0	0-3.0
Mean proliferation fraction by Ki-67	35.4	22.2-48.7	23.6	19.5-27.7
Molecular subtype by IHC				
Luminal A	47.8	29.2-67	73.7	65.3-80.7
Luminal B	0	0-14.3	4.9	2.2-10.3
HER-2 positive	8.7	2.4-26.8	10.7	6.3-17.3
Triple negative	43.5	25.6-63.2	10.7	6.3-17.3

84.3] and lobular cancer: 14.8% [9.5-22.1]. Invasive ductal cancer with mucinous component, invasive cribriform carcinoma, apocrine, atypical medullary, metaplastic, mucinous and invasive ductal breast cancer with papillary components were observed occasionally (Table 1). Although high grade cancers dominated again, proportion of G3 cancers was only 51.6% [41.7-61.3]. However, no statistically significant differences in the grade distribution were observed (Table 1). The p53 expression frequency in CK5/6 positive group was 47.8% [29.2-67.0], but in CK5/6 negative group 24.6% [17.8-32.9]. Both groups lacked actin expression. There was trend towards more frequent p63 expression in CK5/6 positive group; however, p63 protein expression was rare event (Table1). The mean proliferation fraction by Ki-67 was 35.4% [22.2-48.7] in the CK5/6 positive group (Figure 3), but 23.6% [19.5-27.7] in the CK5/6 negative group. Regarding the molecular subtypes, luminal A and triple negative breast cancer were predominant in CK5/6 positive group. Luminal A was the most frequent molecular type in CK5/6 negative tumours constituting even 73.8% [65.0-80.0] of cases.

Evaluating the triple negative group, positive CK5/6 expression was observed in 43.4% [25.6-63.1]. The rate of CK5/6 expression among other molecular types was significantly lower: 10.7% [6.3-17.3] of cases.

Using CK5/6 expression as a complementary marker for triple negative breast cancer, the sensitivity is 43.5% [25.6-63.2], specifity 89.3% [82.6-93.7], positive predictive value 43.5% [25.6-63.2] and negative predictive value 89.3% [82.6-93.7]. Presuming CK5/6 as a marker for basal subtype of triple negative breast cancer, the sensitivity is already set as 100% [72.2-100], but the specifity is 90.4% [84.2-94.3], positive predictive value 43.5% [25.6-63.2] and negative predictive value 100.0% [96.9-100].

DISCUSSION

Breast cancer is a heterogeneous disease. For decades, invasive breast cancers were classified according to histological type (21). More recently, gene expression profiling analysis indicated that breast cancers can be classified into molecular subtypes, including luminal A and B, triple negative or basal-like and HER-2 cases, thereby enabling the prediction of distinct prognosis, clinical outcome and response to therapy (20).

Majority of researchers classify breast cancer by molecular subtype according to DNA microarrays revealing gene expression. However, the gene expression investigation cannot be readily applied to clinically available formalin-fixed, paraffin-embedded tissues constituting the mainstay of diagnostic surgical pathology (17).

Many investigators use IHC to classify tumours but have used different nomenclature. Generally, according to the expression of ER, PR and HER2 protein, breast cancer can be divided in luminal and non-luminal tumours, overall in four groups. If basal markers are added, these four groups can be categorised according to whether or not they express a basal marker yielding a total of eight subtypes. The mapping of these eight IHC subtypes onto the five subtypes based on gene expression is not exact (3). Thus, at present, there is no international agreement on the molecular classification of breast cancer. Although no internationally accepted definition for basal-like cancers exists, the use of CK5 is now well documented and accepted as a useful basal-like tumour marker (22).

The concept of basal-like cancer is based on the normal histology of breast ducts. Normal breast ducts contain at least three types of epithelial cells: luminal (glandular) cells, basal/ myoepithelial cells, and stem cells. Myoepithelial cells typically express high molecular weight cytokeratins like cytokeratin 5/6, 14, 17 as well as p63 protein, actin and CD10 (14).

In reviews where immunohistochemical validation followed after gene expression, basal-like breast cancers can be defined by identifying those tumours that are negative for both ER and HER2 and that are positive for cytokeratin 5/6 and/or HER1 (12). It means that basal-like breast cancer subtype subdivides triple negative breast cancer group but it should be noted that although most basal-like cancers do not express ER or HER-2, 15% to 45% are reported to express at least one of these markers; on the other hand, not all triple negative cancers are of basal-like profile (12, 17, 19). CK5/6 positivity is described in 33% of luminal type carcinomas, and more than a half of HER2 cases can express CK5/6 (15). Similar variability was observed in our study: in CK5/6 positive group luminal A molecular subtype was even slightly more frequent than triple negative breast cancer (47.8% and 43.5%, respectively). In triple negative group, positive CK5/6 expression was found in 43.4% [25.6-63.1], but in the combined group of all other molecular types, 10.7% [6.3-] of cases.

In the present study, CK5/6 as a complementary marker for triple negative breast cancer shows low sensitivity although sufficient specifity. Thus, CK5/6 positive breast cancer is not synonymous with triple negative breast cancer in accordance with Blows *et al.* although strongly associated with this molecular type (3).

Age is one of the most important validated prognostic factors for breast cancer (6). The characteristic features of triple-negative breast cancer include earlier age of onset along with worse outcome, higher incidence of visceral and brain metastases, higher incidence in African - American women, predominance in pre-menopausal women, and a distinct mechanism of hematogenous metastatic spread (18). The mean age in our study in CK5/6 positive and negative group was practically equal. In comparison to other basal breast cancer studies, our value is similar or slightly higher (2, 9, 20).

Triple-negative breast cancer is histologically characterized by high grade, high proliferative rate, central necrosis, apoptotic cells and lymphocytic response. By morphology triple negative breast cancers mostly are high grade infiltrating ductal carcinomas, high grade infiltrating lobular carcinomas, high grade metaplastic myoepithelial carcinomas, apocrine and medullary tumours (15, 18). The expression of basal markers has been reported in 2-18% of ductal invasive carcinoma cases, and in 25% of G3 breast carcinoma (15). Metaplastic or anaplastic carcinomas, widely recognized as tumours with an unfavourable prognosis, are basal-like breast cancers. Histological grade is significantly associated with molecular subtypes. Basal-like breast cancer and HER-2 positive breast cancer showed the highest prevalence of high grade phenotype (71.1-82% and 64-70% in different researches respectively) whereas luminal A tumours were more frequently well/ moderately differentiated (84.6-89%) (15, 20). In our study invasive ductal breast cancer with predominance of high grade cases composed the largest group of CK5/6 positive breast cancer. However, CK5/6 negative group also contained high proportion of ductal breast cancer, the most common histological type of breast cancer. Thus, the histological characteristics of CK5/6 positive breast cancer correspond to the published findings but lack specifity.

p53 is tumour suppressor protein. A mutation or deletion of the respective gene is frequently observed in many aggressive tumours. Over-expression and mutation of p53 are seen in 82% to 85% of basal-like breast tumour (5, 18). In contrast, mutations of p53 were present in 13% of luminal A breast cancer cases (18). With regards to refining the immunohistochemical classification of basal-like breast cancer, the clinical utility of p53 in routine analysis may be limited since the specific location and type of mutation in the protein was recently shown to influence clinical outcome in breast cancer patients. Consequently, while p53 accumulation is considered a classic indicator of its mutation status, the best approach for p53 analysis is likely a more technically demanding combination of immunohistochemistry and genetic screening techniques as recently illustrated by Manie *et al.* (11). In our study, there was a trend towards more frequent p53 expression in CK5/6 positive group. However, the difference did not reach statistic significance. This can be at least partially attributed to the above mentioned biological and technical factors.

p63 is a member of the p53 gene family and is involved in cellular differentiation. It is expressed in the nuclei of myoepithelial cells of normal breast. p63 is strongly expressed in metaplastic carcinoma (86.7-100%), but only in 0.6% of non-metaplastic invasive breast carcinomas (8). In our study, expression of p63 was a rare event, partially due to low numbers of metaplastic breast cancer. However, CK5/6 positive tumours showed a trend towards more frequent p63 expression not limited by metaplastic breast cancer histology. Expression of actin, another classic myoepithelial marker was not found in our study. This partially contrasts with the described immunoreactivity for actin in up to 22% of the basal-like tumours, but none of the luminal or HER-2 positive tumours (10). However, there is a trend towards more frequent occurrence of other basal markers in CK5/6-positive group.

The expression of cell cycle-characterizing Ki-67 protein expressed as Ki-67 index (percentage of positive cells) is widely used in the routine assessment of prognostic markers. Nevertheless, it is not considered a standard due to the lack of an international standardization method for antigen retrieval, staining procedures and semi quantitative and quantitative scoring methods (6). St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer in 2009 provided the assessment scheme of Ki67-labelling index in which Ki-67 is considered low if count of proliferative cells is below or equivalent with 15%, intermediate within the limits from 16% to 30% and high exceeding 30% (7). High Ki-67 index is associated with the basal-like subtype and poor prognosis (5, 12). In our study, the mean proliferation fraction by Ki-67 was 35.4% in the CK5/6 positive group, but 23.6% in the CK5/6 negative group showing trend towards higher proliferative activity in CK 5/6 positive group.

CONCLUSIONS

1. By immunohistochemistry, CK5/6 expression is found in 15.9% of breast cancers. Frequency of this parameter is sufficient to carry out the clinical examination.
2. CK 5/6 expression is not limited to single molecular type. Luminal A and triple negative breast cancer constitute the largest groups within CK5/6 positive cases.
3. CK5/6 positive tumours tend to have higher proliferation fraction.
4. In triple negative group CK5/6 expression is significantly more frequent than in other molecular types. However, CK5/6 as a complementary marker for triple negative breast cancer is characterised by low sensitivity although high specifity.

Conflict of interest: None

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ORIGINAL ARTICLE

Prognostic Factors in Potentially Radically Operated Pancreatic Carcinoma

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Summary
Introduction. About 300 new cases of pancreatic carcinoma are diagnosed in Latvia every year. Despite the best available treatment, the prognosis of pancreatic cancer is still poor, as over 90% of the patients die within 1 year of diagnosis.
Aim of the Study. To investigate the frequency of known prognostic factors in potentially radically operated pancreatic carcinoma in Latvian patients' in order to reveal the intrinsic tumor biology.
Materials and methods. The study was performed as a retrospective investigation. Forty-nine consecutive cases were identified by archive search in Pauls Stradiņš Clinical University Hospital, 2004 – 2010. The histogenesis and pTNMGR parameters of the carcinoma were evaluated. For the data analysis, descriptive statistics was performed by CIA software involving 95% confidence interval (CI) analysis.
Results. The mean age of the patients was 63.1 years (95% CI = 60.0 – 66.2). In 43/49 (87.8%; 95% CI = 75.8 – 94.3) cases the tumor was larger than 2 cm. High frequency of T3 (44/49; 89.8%; 95% CI = 78.2 – 95.6) was identified. Metastases in regional lymph nodes were detected in 29/49 (59.2%; 95% CI = 45.2 – 71.8) cases. Metastases were detected in 33% (95% CI = 15.2 – 58.3) of cases when less than 6 lymph nodes were examined, but the frequency of lymph node metastases was 61.5% (95% CI = 35.5 – 82.3) when 6 – 11 lymph nodes were examined. The most frequent stage of the tumor was IIB (26/49; 53.1%; 95% CI = 39.4 – 66.3). Resection margins were involved in 18/49 (36.7%; 95% CI = 24.7 – 50.7) of all cases. Perineural invasion was the most common manifestation of invasion, found in 37/49 (75.5%; 95% CI = 61.9 – 85.4) cases.
Conclusions. Pancreatic carcinoma is characterized by frequent presence of potentially unfavorable factors such as high T, presence of metastases in lymph nodes, perineural invasion and high tumor grade (G3). Considering the frequency of R1 that was discovered in our study, the resection margin should be well distanced from the tumor.
Key words: pancreatic carcinoma, TNM, perineural growth.

INTRODUCTION

Pancreatic carcinoma is an aggressive disease. The number of patients that are registered in the Centre of Health Economics with the diagnosis of pancreatic carcinoma is growing. In year 2007 there were 360 registered patients with pancreatic carcinoma, but in year 2010 - 405 patients (http://vec.gov.lv; accessed 15.08.2011.). Surgical treatment of this carcinoma is very difficult and the surgeon's skills have to be very proficient. Furthermore, the operation is quite often not possible because of the spread of tumor or the condition of the patient. Therefore, despite using the best available treatment methods, the prognosis of pancreatic cancer is still poor. Five year survival in non-surgically treated pancreatic carcinoma patients is 4%, but it reaches 15% after a radical resection of the tumor (Rosai, 2004). Several prognostic factors (Table 1) affect patients' prognosis after the operation (Lim *et al.*, 2003).

AIM OF THE STUDY

Aim of the study is to investigate the frequency of known prognostic factors in potentially radically operated pancreatic carcinoma in Latvian patients' in order to reveal the intrinsic tumor biology.

MATERIALS AND METHODS

The study was performed as a retrospective investigation. Forty-nine consecutive cases were identified by archive search in Pauls Stradins Clinical University Hospital during time period 2004 – 2010. Sex and age of the patients were recorded. Technique of the operation, localization of the tumor and the number of operations per year was included in the characteristics of the surgery. Data for the tumor characteristics were obtained by analysis of the surgical pathology reports (gross measurements) and diagnostic pathology slides. The evaluated histopathologic factors were following: size of the tumor by the largest diameter, T stage (T), lymph node status (N), presence of distant metastasis (M), anatomic stage, histologic grade (G), vascular, perivascular, perineural and intraneural invasion, status of resection margins (R), mitotic activity per 10 high power fields (HPF). The selection of prognostic factors was based on literature studies (Table 1). For the data analysis, descriptive statistics involving 95% confidence interval (CI) calculation was performed by CIA software (Altman *et al.*, 2000).

Table 1. Prognostic factors in relation to the overall survival for pancreatic adenocarcinoma

Prognostic factors	Median survival (months)	1 year survival (%)	2 year survival (%)	3 year survival (%)	5 year survival (%)	References
Stage IA	24.1	71.3	50.2	40.7	31.4	Edge *et al*, 2010
Stage IB	20.6	67.3	45.4	35.3	27.2	
Stage IIA	15.4	60.7	34.9	23.8	15.7	
Stage IIB	12.7	52.7	23.8	14.4	7.7	
Stage III	10.6	44.5	19.3	11.0	6.8	
Stage IV	4.5	19.2	8.4	5.3	2.8	
T1 and T2	19.9	66.2	-	-	-	Lim *et al*, 2003
T3	17.8	59.0	-	33.1	-	
Tumor diameter equal or less than 2cm	37.8	75.7	-	51.3	-	
Tumor diameter more than 2cm	14.8	55.2	-	28.8	-	
N0	19.9	64.0	-	37.9	-	
N1	15.5	56.0	-	29.1	-	
Low histologic grade	35.3	76.4	-	51.8	-	
Moderate histologic grade	14.8	56.7	-	31.0	-	
High histologic grade	12.5	51.8	-	26.8		
Invasion in large blood vessels	-	-	-	-	8	Allema *et al*, 1995
Negative margins of resection	-	-	-	-	49	
Positive margins of resection					4	
Patients' age less than 65 years	-	-	-	-	8.5	http://seer.cancer.gov/
Patients' age more than 65 years	-	-	-	-	3.6	

RESULTS

Characteristics of the patients. There were 49 patients undergoing potentially curative resection of pancreatic carcinoma, 2004 – 2010. The study group included 22 men and 27 women. The age of the patients ranged 39 – 81 years, mean 63.1 years (95% CI = 60.0 - 66.2). Most frequently, women (7/27; 25.9%; 95% CI = 13.2 – 44.7) were 66 – 70 years old at the time of operation, but men (6/22; 27.3%; 95% CI = 13.2 – 48.2) were 56 – 60 years old (Figure 1). In 25 of 49 cases (51.0%; 95% CI = 37.5 – 64.4), including 16/27 women (59.3%; 95% CI = 40.7 – 75.5) and 9/22 men (40.9%; 95% CI = 23.3 – 61.3), the patients were older than 65 years.

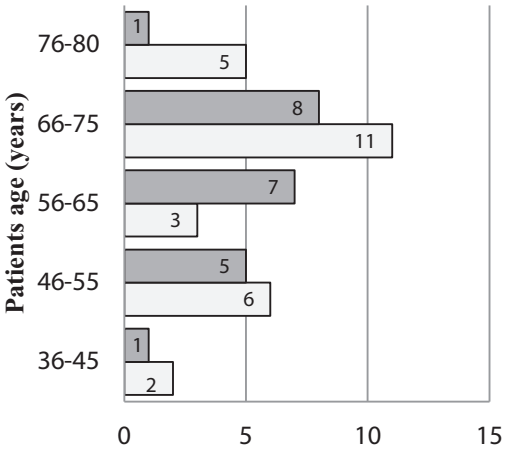

Fig. 1. Patients' characteristics by age and gender.

Characteristics of the surgical approach. Pancreaticoduodenectomy (Whipple procedure) was performed in 37/49 (75.5%) cases, total pancreatectomy in 5/49 (10.2%) cases, distal pancreatectomy in 6/49 (12.3%) cases and Beger procedure in 1/49 (2.0%) case. In 6 cases splenectomy also was performed; in 4 cases along with total pancreatectomy and 2 – with distal pancreatectomy. Liver metastases were resected in 2 cases.

Pancreatic carcinoma was localized in the head of the pancreas in 41/49 (83.7%) cases; in the body of the pancreas in 4/49 (8.2%) cases; showed wide spread throughout the body and the tail in 1/49 (2.0%) case and was situated in the tail of the pancreas in 3/49 (6.1%) cases. There were 12 operations in the year 2010, 8 operations in 2009 and 2007, 7 operations in 2008 and 2006, but only 3 in 2005 and 4 operations in 2004.

Histopathologic characteristics. The tumor was pancreatic exocrine carcinoma in 45/49 cases (91.8%; 95% CI = 80.8 – 96.8) including a single case of *carcinoma in situ*. The other exocrine carcinomas were represented by invasive ductal adenocarcinoma. Pancreatic neuroendocrine carcinoma constituted 4/49 (8.2%; 95% CI = 3.2 – 19.2); 2 tumors were localized in the tail and 2 in the body of the pancreas.

In only 6 of 49 cases (12.2%; 95% CI = 5.7 – 24.2) the largest diameter of the carcinoma was 2 cm or less. Analyzing T stage, the following data were obtained. *Carcinoma in situ* (Tis) constituted 1/49 (2.0%; 95% CI = 0.4 – 10.7). There were no T1 carcinomas, but there was 1/49 (2.0%; 95% CI = 0.4 – 10.7) T2 case. The vast majority, namely 44/49 (89.8%; 95% CI = 78.2 – 95.6), carcinomas corresponded to T3, but there were 3/49 cases (6.1%; 95% CI = 2.1 – 16.5) of T4 spread. No regional lymph node metastases (N0) were found in 18/49 (36.7%; 95% CI = 24.7 – 50.7) cases. The regional lymph nodes were not assessed by the pathologist (Nx) in 2/49 (4.1%; 95% CI = 1.1 – 13.7) patients. The lymph node metastases (N1) were present in 29/49 (59.2%; 95% CI = 45.2 – 71.8) cases (Figure 2). The detection of these metastases depends on the number of examined

lymph nodes (Figure 3). If less than 6 lymph nodes were evaluated, the rate of N1 was 33.3% (95% CI=15.2 – 58.3). In contrast, the rate of metastases was 61.5% (95% CI=35.5 – 82.3) in cases where 6 – 11 lymph nodes were examined, 72.2% if 12 – 23 lymph nodes were detected and 100% when more than 23 lymph nodes were detected.

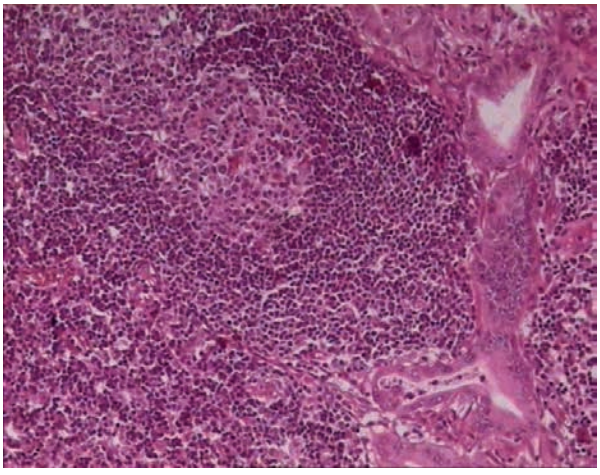

Fig. 2. Regional lymph node metastasis of ductal adenocarcinoma of the pancreas. Hematoxylin – eosin, original magnification 100x.

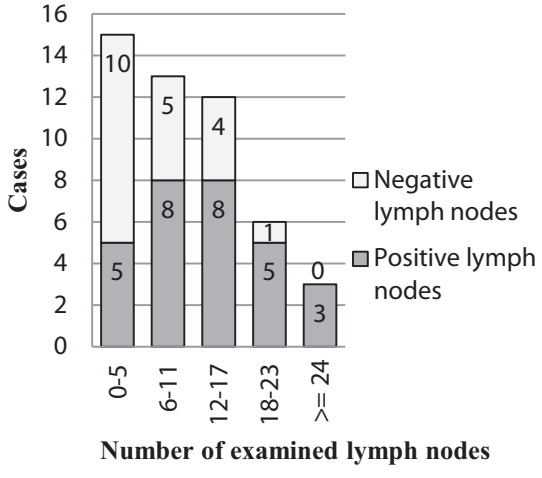

Fig. 3. Amount of metastatic lymph nodes by quantity of examined peripancreatic lymph nodes in potentially radically operated pancreatic cancer cases.

A distant spread of tumor was proved in 2/49 (4.1%; 95% CI = 1.1 – 13.7) patients. In both cases liver metastases were resected. In 1 of 49 cases (2.0%; 95% CI = 0.4 – 10.7) stage 0 was detected. There were no cases in stages IA and IB. Stage IIA was detected in 16/49 (32.7%; 95% CI = 21.2 – 46.6) cases and IIB in 26/49 (53.1%; 95% CI = 39.4 – 66.3) cases. In 2/49 cases substage was indefinable, because the lymph nodes were not assessed. Stage III as well as stage IV was established in 2/49 (4.1%; 95% CI = 1.1 – 13.7) cases, respectively.

Considering the grade of the tumor, 10 of 49 cases (20.4%; 95% CI = 11.5 – 33.6) were low grade (G1) tumors (Figure 4). In 19 of 49 cases (38.8%; 95% CI = 26.4 – 52.8), the patients had developed moderate grade (G2) tumors and 20/49 (40.8%; 95% CI = 28.2 – 54.8) cancers were high grade (G3) tumors (Figure 5).

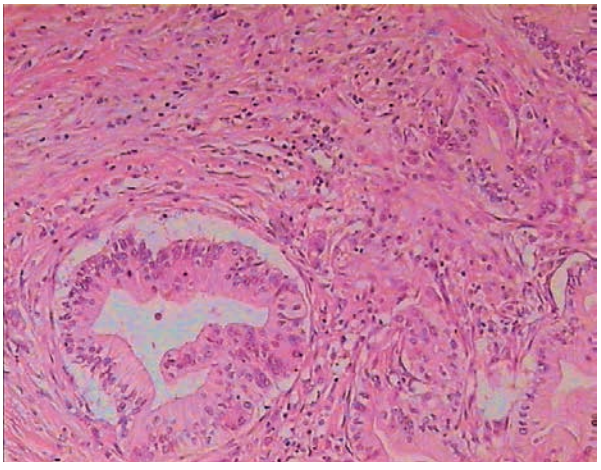

Fig. 4. Low grade ductal adenocarcinoma of the pancreas. Hematoxylin – eosin, original magnification 100x.

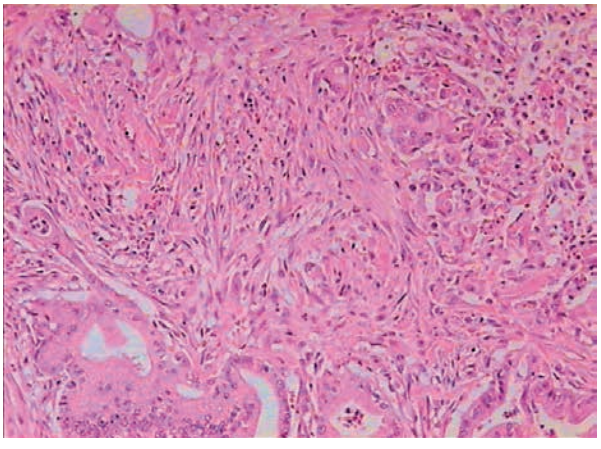

Fig. 5. High grade ductal adenocarcinoma of the pancreas. Hematoxylin – eosin, original magnification 100x.

In 7/49 (14.3%; 95% CI = 7.1 – 26.7) cases resection margins have not been assessed by the pathologist (Rx). In 24/49 (49%; 95% CI = 35.6 – 62.5) cases resection lines were not involved by the tumor (R0), but in 18/49 (36.7%; 95% CI = 24.7 – 50.7) cases the resection margins were positive (R1).

The number of mitoses ranged 0 – 11, mean 4.9 (95% CI = 4.0 - 5.8). In 3/49 (6.1%; 95% CI = 2.1 – 16.5) cases the tumor invaded large blood vessels, in 16/49 (32.7%; 95% CI = 21.2 – 46.6) cases - small blood vessels. Perivascular invasion was revealed in 3/49 (6.1%; 95% CI = 2.1 – 16.5) cases. In 37/49 (75.5%; 95% CI = 61.9 – 85.4) cases perineural growth was detected. Intraneural spread was found in 11/49 (22.4%; 95% CI = 13.0 – 35.9) cases (Figure 6).

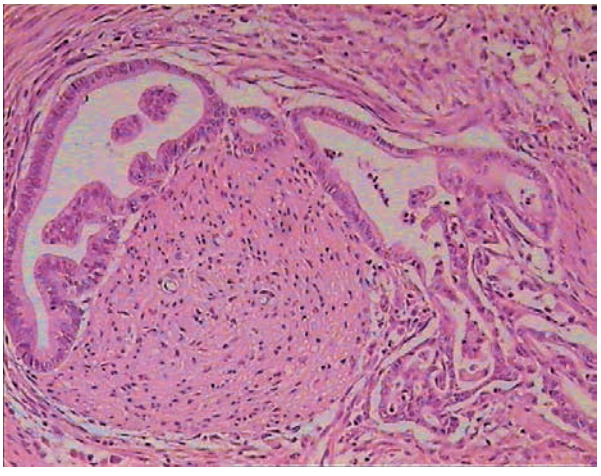

Fig. 6. Perineural invasion of ductal adenocarcinoma of the pancreas. Hematoxylin – eosin, original magnification 100x.

DISCUSSION

Potentially radical surgery plays the key role in the treatment of pancreatic carcinoma as it has been associated with increased survival (Lim *et al.*, 2003). However, the prognosis after the operation still remains serious. We performed the present study in order to reveal the frequency of several prognostic factors that would be useful for comprehensive analysis of the disease course in Latvian patients. Only potentially radically operated patients were included in the study as this condition provides the possibility to confirm the diagnosis with certainty.

The pancreatic carcinoma usually is diagnosed at the age of 60 – 80 years. Cases below the age of 40 years are rare (Kl ppel *et al.*, 2000). The patients that were included in the present study were 39 – 81 years old. In our study 25 of 49 patients (51.0%; 95% CI = 37.5 – 64.4) were more than 65 years old, which is a poor prognostic factor by itself (http://seer.cancer.gov/; accessed 02.01.2011). The mean age of 63.1 years (95% CI = 60.0 – 66.2) in our group is statistically different from the value found by a large study of the prognostic factors in pancreatic adenocarcinoma, where the mean age was 72 years (Lim *et al.*, 2003). It could be because of differences in the duration of the study, as well as the differences in population, genetic structure and environment.

In the time period 2004 – 2010 the number of performed operations increased, reflecting the improvements in the diagnostics and the development of medicine in general. Since the most frequent localization of the carcinoma was in the head of the pancreas (84%), Whipple procedure was performed most frequently (75.5%). In 8.2% cases the carcinoma was localized in the body of the pancreas, which is less than it is described in literature – 15% (Hruban and Iacobuzio-Donahue, 2010). The difference can be attributed to the set limits – only patients for whom potentially radical surgery was possible were included in the study. Regarding other aspects of surgery and tumor localization, there were no significant differences between our study and the data from the literature.

Histopathologically factors that worsen the prognosis include metastatic disease in regional lymph nodes, high grade histology, perineural invasion and larger size of the primary tumor (Edge *et al.*, 2010). The prognosis also differs between pancreatic adenocarcinoma and neuroendocrine carcinoma. Patients who undergo surgical resection for non-metastatic adenocarcinoma of the pancreas have a 5-year survival of 20%, but patients with neuroendocrine carcinoma – 55.4% (Edge *et al.*, 2010). In our study 91.8% (95% CI = 80.8 – 96.8) had pancreatic adenocarcinoma similarly to the published findings, namely 85% from pancreatic malignancies (Rosai, 2004). In 8.2% (95% CI = 3.2 – 19.2) cases neuroendocrine carcinoma was found. The published data reflecting frequency of neuroendocrine carcinoma are controversial. Edge *et al.* has described that the frequency of neuroendocrine carcinoma is 3 – 5% of all pancreatic malignancies (falls within 95% CI of our study), but in the DeLellis *et al* study it was significantly lower: 1 – 2% (Edge *et al.*, 2010; Heitz *et al.*, 2004).

If the size of the tumor is more than 2 cm, it is considered a prognosis worsening factor. In our study the size of the tumor exceeded 2 cm in 87.8% (95% CI = 75.8 – 94.3) cases. Similar value of 77.3% is reported by Lim *et al.*, 2003. The diagnosis in most cases is made when the tumor is relatively large (5 cm). Carcinoma of the head of the pancreas causes progressive jaundice that is associated with pain in at least half of the patients (Rosai, 2004). Carcinomas of the body and tail of the pancreas do not involve bile ducts; therefore, the diagnosis is made even later, the tumor is larger and the spread is wider, reaching stages T3 and T4 (Hruban and Iacobuzio-Donahue, 2010). In our study most tumors were in stage T3 (89.8%; 95% CI = 78.2 – 95.6) in accordance with other studies – 81% (Lim *et al.*, 2003). Metastases were found frequently (59.2%; 95% CI = 45.2 – 71.8), but this number could be even larger, because in 57.1% of cases insufficient number of lymph nodes has been investigated (a specimen should include analysis of a minimum of 12 lymph nodes). The number of the examined lymph nodes is very important and the results show that if less than 6 lymph nodes were examined, only 33.3% were positive, but if 12 – 23 lymph nodes were examined, then 72.2% were positive. The anatomic stages/prognostic groups of the tumor are defined by summarizing the data about TNM parameters. Most frequently the stage of the tumor was IIB (53.1%; 95% CI = 39.4 – 66.3). In 32.7% (95% CI = 21.2-46.6) cases stage IIA was observed. Since these

stages differ by N0 or N1, the proportion of these stages could likely be affected by Nx cases. Although in other studies IIB and IIA are also the most frequent stages, there is a statistically significant difference between our results and Lim *et al.*, 2003, where stages IIB and IIA were less frequent (36.4%; 17.9%), but stage IV was observed in 12.7% of cases (Lim *et al.*, 2003). In our study only 4.1% (95% CI = 1.1 – 13.7) cases were in stage IV.

The status of resection margin is an important prognostic factor. In our study resection margins were not assessed by the pathologist (Rx) in 14.3% (95% CI = 7.1 – 26.7) cases. Such a large number of Rx should be reduced, because if the resection margins are positive, 5 year survival is the same as in non-surgically treated patients, resp., 2 – 4% (Allema *et al.*, 1995; Edge *et al.*, 2010). Positive resection margins occurred in 36.7% (95% CI = 24.7 – 50.7) cases in our study.

High histologic grade also decreases the survival. Ductal adenocarcinoma of the pancreas mostly had high or moderate grade – 40.8% (95% CI = 28.2 – 54.8) and 38.8% (95% CI = 26.4 – 52.8), respectively. All neuroendocrine carcinomas in the study group were well differentiated thus associated with a better prognosis. Mitotic count is included in the criteria of the tumor histologic grade. The mitotic activity was lower in the cases of our study than it is described in the literature for the corresponding tumor grades. However, it should be taken into account that glandular differentiation, mucin production and nuclear features (Luttges *et al.*, 2000) are also important factors in the evaluation of the histologic grade of the ductal adenocarcinoma.

When evaluating the invasion of the tumor, perineural spread was the most frequent (75.5%; 95% CI = 61.9 – 85.4), which is described as a survival decreasing factor (K ppel *et al.*, 2000). Invasion in small blood vessels and intraneural invasion was also frequently found. This type of invasion of the pancreatic carcinoma is frequently the cause of an incomplete resection (Edge *et al.*, 2010).

CONCLUSIONS

1. Pancreatic carcinoma is characterized by a frequent presence of potentially unfavorable factors, such as high T, presence of metastases in lymph nodes, perineural invasion and high tumor grade (G3).
2. Examination of less than 6 peripancreatic lymph nodes is associated with lower rate of N1.
3. Prognostically important factors can be identified in the material extracted during the resection of a pancreatic carcinoma. For the complete evaluation it is advisable to establish a diagnostic protocol that would allow the elimination of Rx and insufficient examination of lymph nodes.
4. Considering the frequency of R1 that was discovered in our study, the resection margin should be well distanced from the tumor.

Conflict of interest: None

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ORIGINAL ARTICLE

Immunocytochemistry as an Adjunct to Fine-needle Aspiration of Thyroid in Distinction Between Benign and Malignant Thyroid Neoplasms

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Summary
Introduction. Thyroid nodules are very common therefore distinction between benign and malignant tumors is essential for proper clinical management.
Aim of the study. The study was performed to evaluate the diagnostic value of molecular markers in different thyroid tumors.
Materials and methods. Forty-eight thyroid FNA cases confirmed by subsequent surgical resection specimens were selected. Immunocytochemistry for HBME-1, CD56 and E-cadherin (E-CAD) was performed. The study group consisted of 15 papillary thyroid cancers (PTC) and 1 follicular carcinoma (FC) as well as 12 follicular adenomas (FA) and 20 cases of colloid goiter (CG).
Results. The expression of HBME-1 in PTC was significantly higher than in another thyroid lesions. E-CAD and CD56 expression was found in 8/12 (66.6%) and 6/12 (50%) cases of FA respectively. In contrast, PTC showed very low expression (1/15) of both E-CAD and CD56. Expression of the three analyzed markers was not more than 10% in case of CG.
Conclusions. We concluded that immunocytochemical (ICC) staining is of value as an ancillary test to enhance the diagnostic accuracy of thyroid FNA biopsies. Larger studies dedicated to evaluate the role of these or other markers for distinction between FC and FA can be particularly useful. We recommend the small panel consisting of three ICC markers, HBME-1, E-CAD and CD56 as an adjunct to standard cytomorphology criteria to enhance the diagnostic accuracy of thyroid nodules with follicular-patterned cytologic features.
Key words: immunocytochemistry, thyroid nodules, HBME-1, CD56, E-cadherin.

INTRODUCTION

Thyroid nodules are very common and till now many studies evaluating diagnostic criteria for differentiating benign from malignant nodules have been reported. It is estimated that 5% of the general population develops clinically palpable thyroid nodules and with the emergence of ultrasound (US), impalpable thyroid nodules can be detected in 20–67% of the general population (12, 38). Accurate diagnosis of thyroid nodules is critical for proper clinical management. Thyroid cancer represents ~5–24% of thyroid nodules and ~1–2% of all malignancies (19) were papillary thyroid carcinoma (PTC) constitutes about 80% of all thyroid malignancies (11, 30).

Differential diagnosis of thyroid nodules could be difficult due to overlapping morphological features and as a result, up to 85% of patients with suspicious cytology who subsequently undergo surgery have benign lesions (17).

Management of the thyroid nodules should be guided by the US evaluation in combination with fine-needle aspiration (FNA) biopsy. FNA is the first choice for diagnostic evaluation of thyroid nodules larger than 1 cm in diameter or nodules with suspicious findings on transcutaneous US. It is an accurate, reliable, and simple procedure to perform, and nowadays, is accepted as a standard diagnostic method for the differential diagnosis of thyroid nodules (23, 21). Usually US guided FNA is performed to reduce the rate of nondiagnostic biopsies (40). Contraindications are very few and complications such as hematoma or infections are rare. Among other preoperative tests it has been shown to be a better predictor of malignancy and has decreased the number of thyroid operations by about half together increasing the yield of cancer from 15-50% (25).

The use of FNA to procure tissue for microscopic diagnosis is almost 100 years old. In 1904 Greig and Grey reported that trypanosomal organisms could be detected in needle aspiration material from lymph nodes in a patient with sleeping sickness (15, 39). During the 1960s FNA became a standard procedure in Sweden not only for the thyroid but also for all palpable lumps in the body (39).

According to recent available guidelines FNA of the thyroid cytological diagnoses should be organised into five categories: benign, nondiagnostic, follicular lesion, suspicious for malignancy and malignant (13). In several large series FNA results are classified as benign in 60-80%; nondiagnostic in 10-15%; follicular lesions in 10-20%; suspicious in 2.5-10% and 3.5-10% are malignant (41, 31). Nevertheless, FNA is limited by sampling difficulties that result in "nondiagnostic"

aspirates and by significant overlap in morphologic features between benign and malignant nodules it can result in inconclusive result (24).

Immunohistochemistry (IHC) was introduced to the practice of pathology in the early 1970s, but in thyroid pathology, its use has been restricted to differential diagnosis between follicular and C-cell derived neoplasms. Nowadays a growing number of detected molecular markers, regardless of the presence of capsular or vascular invasion, have provided interesting insights into the role of immunohistochemistry in thyroid neoplasms (10, 34, 6, 20).

Our group of researchers recently published a study about applying the panel of IHC markers on histological samples of benign and malignant thyroid lesions (30). In our opinion immunocytochemistry (ICC) may find new application in aspiration cytology because this technique has been recently introduced to obtain thin layer slides in cervical cytology. Excellent results in this field have prompted a wider application to almost all cytological branches, including thyroid FNA (32). The current study of panel comprising three molecular markers (HBME-1, E-Cadherin (E-CAD), CD56) was performed to evaluate its effectiveness in discriminating between benign and malignant thyroid lesions on thin layer slides.

MATERIALS ANS METHODS

Forty-eight thyroid FNA cases confirmed by subsequent surgical resection specimens, during the period of 2010-2011, were selected from the Institute of Pathology, Pauls Stradins Clinical University Hospital, Riga, Latvia. The study group consisted of 16 malignant and 32 benign thyroid lesions including 15 PTC and 1 follicular carcinoma (FC) as well as 12 follicular adenomas (FA) and 20 cases of colloid goiter (CG).

The institutional Committee of Ethics approved the study.

Fine needle aspiration procedure was performed by experienced radiologist using GE Voluson E8 ultrasound machine and 11L-D linear transducer. During FNA procedure patients were placed in supine position, the puncture site was prepared sterile and draped. US probe was covered by condom and disinfected with Cutasept F solution. Local anesthetic 1.0 ml Lidocaine 20 mg/ml was used. A 21-gauge needle was attached to 20 mL syringe. Under real-time visualization needle tip was introduced in suspicious nodules. Passes were done using 5-10 ml suction. A minimum of 2 passes was employed. Needle placement was documented by taking pictures. Aspirated material was placed, smeared on Histobond adhesive glass slides and air-dried.

Immunocytochemistry. For immunophenotypic studies the cell smears were air dried and fixed in 96% ethanol for 10 min. Endogenous peroxidase activity was blocked by 0.5% hydrogen peroxide in methanol for 10 min. All chemicals were produced by Sigma-Aldrich (Steinheim, Germany). After rinsing in TBS buffer (pH 7.6, Tris-buffered saline, THAM-HCl 50 mM/l, NaCl 150 mM/l) for 5 min, the slides were encircled with Dako pen (Dako, Glostrup, Denmark) and transferred to magnetic immunostaining trays (CellPath plc, Newtown, UK). After the rinse with TBS buffer for 5 min, the incubation with primary antibodies was carried out at room temperature for 60 min as described [30]. Unbound primary antibodies later were removed by repeated rinses with TBS buffer 2x 5 min. A commercially available polymeric EnVision+ System, bound with horseradish peroxidase (Dako), was used for visualization. The slides were incubated in a humid chamber for 30 min with EnVision+ with following rinses in TBS 2x5 min. The color development was obtained with 3,3-diaminobenzidine (Dako) for 10 min. The slides then were rinsed in water and counterstained in haematoxylin for 3 min. Positive and negative control slides were included in each run.

The statistical evaluation of the data was carried out using the Statistical Package for Social Sciences (SPSS® version 17.0) and Microsoft Excel programs. The statistical significance was determined by 95% confidence interval. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated.

RESULTS

The results of immunocytochemical staining in benign and malignant thyroid lesions are summarized in Table 1. The expression of HBME-1 in PTC was significantly higher than in another thyroid lesions included in the study. HBME-1 expression was absent in benign lesions as well as in the only case of FC. The expression of E-CAD (Fig. 1) and CD56 (Fig. 2) was found in 8/12 (66.6%) and 6/12 (50%) cases of FA respectively. In contrast, PTC showed very low expression (1/15) of both E-CAD and CD56. Expression of the three analyzed markers was not more than 10% in case of CG. HBME-1 has an extremely high value in the differential diagnostics of PTC showing high ability to discriminate between PTC and FA or benign tissues.

To distinguish benign thyroid tissue from malignant using FNA combined with ICC and taking into account the presented results, the sensitivity, specificity, PPV and NPV were calculated. The sensitivity and specificity of the method was 87.5% and 100%, respectively; the PPV-100% and NPV-88.8%.

DISCUSSION

Thyroid nodules are common lesions in clinical practice and during the last decade, there have been increasing attempts to find additional criteria to make the diagnosis of thyroid nodules more accurate.

As early as 1982 it was stated that FNA is the most sensitive and specific test for diagnosis of thyroid nodules. FNA is the first-line tool for preoperative diagnosis of nodules in the thyroid, and most publications report excellent results (1, 2, 29, 28).

FNA has two major limitations: nondiagnostic results and suspicious results. The rate of nondiagnostic cytological results varies from 2-21% (16). Considering this result, nondiagnostic cytologies might not be of benign cytology

and should be evaluated carefully. Nevertheless, there are some inherent limitations in thyroid FNA, and cytopathologist sometimes still come across difficulties in rendering accurate diagnosis. In fact, one frequent dilemma that faces cytopathologist is the inability to differentiate benign from malignant follicular tumors, because the distinction is based strictly on the presence or absence of vascular and or capsular invasion (3, 33). Another problem is distinguishing between follicular variant of PTC and follicular neoplasm on thyroid FNA samples (22, 36). Up to now, no effective method has been established to accurately distinguish these thyroid lesions in the pre surgical phase.

During the last decade, there have been increasing attempts to find additional criteria to make more accurate diagnosis of thyroid nodules. Studies have been conducted mostly on surgically resected thyroid specimens to evaluate the utility of various molecular markers however, similar studies were also conducted on FNA cytological specimens using different kind of cell fixation techniques (27, 34). We chose these most promising markers (HBME-1, CD56, E-CAD) on a histologically proven thyroid lesions and test them on FNA biopsies of benign and malignant thyroid lesions.

Anti-HBME-1, first described in 1992 by Battifora et al., is a monoclonal antibody directed against an antigen of the microvillous surface of mesothelioma cells [34]. In several studies on tissue samples and on FNA products, it has been found positive in thyroid malignancies, especially in PTC (30, 6, 20).

According to our data, HBME-1 had a high immunoexpression level in PTC. There was no HBME-1 immunoexpression in benign lesions. This agrees with the study by Nasr et al. (26) showing positive expression of HBME-1 in 49/51 (96%) of PTC, whereas normal thyroid tissue was consistently negative. A study by Saleh et al. (33) concluded that HBME-1 has high sensitivity and specificity of immunoexpression in all benign lesions versus malignant tumors, 88.9% and 72.7%, respectively. Several recent investigators have reported high HBME-1 expression in PTC and some FC, but low or negative expression in FA (27, 34, 26).

E-CAD is transmembrane glycoprotein, which is present in most epithelial cells and appears to play an important role in the development and progression of human carcinomas (18). The expression of E-CAD in normal thyroid was first described by Eidelman et al. (7). Since that, several studies have investigated the expression of E-CAD in thyroid malignancies. It is recognized that the reduction of E-CAD expression is associated with thyroid neoplasms (5, 14). Analyzing our data, only 1/14 (6.66%) PTC showed E-CAD expression when compared to FA 8/12 (66.6%). CG also showed low expression of E-CAD 2/20 (10%). In contrast, FC showed no E-CAD expression. The low and heterogenous expression of E-CAD in PTC confirms the results of Soares et al. (37) and Brabant et al. (4), who observed a reduction of E-CAD in a PTC compared to normal thyroid tissue.

CD56 is a neural cell adhesion molecule that is present on follicular epithelial cells of the normal thyroid (42). Its expression may affect the migratory capability of tumor cells (8, 9). Our results showed positive CD56 immunoexpression in 6/12 (50%) FA and extremely low expression in CG and PTC. CD56 was not detectable in single case of FC. Overall these findings are similar to the results reported by Scarpino et al. showing low or absent expression of CD56 in PTC and FC using immunohistochemistry (35). Further, El Demellawy et al. showed CD56 as extremely useful in the distinction between PTC and follicular lesions or neoplasms. According to their results, diffuse CD56 expression was present in normal and neoplastic follicular epithelium, but it was not significant in PTC (8, 9).

CONCLUSIONS

We concluded that ICC staining is of value as an ancillary test to enhance the diagnostic accuracy of thyroid FNA biopsies. However, ICC must be used along with, and not to stand in the place of well established cytological criteria. Unfortunately, in our study there is only one case of FC. Larger studies dedicated to evaluate the role of these or other markers for distinction between FC and FA can be particularly useful. We recommend the small panel consisting of three ICC markers, HBME-1, E-CAD and CD56 as an adjunct to standard cytomorphology criteria to enhance the diagnostic accuracy of thyroid nodules with follicular-patterned cytological features.

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Conflict of interest: None

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Table 1. Descriptive statistics of marker expression in different thyroid lesions

	FA (12)		CG (20)		PTC (15)		FC (1)	
Marker	Expression n (%)	95% CI	Expression n (%)	95% CI	Expression n (%)	95% CI	Expression n (%)	95% CI
HBME-1	0 (0%)	0-30.1	0 (0%)	0-20.0	14 (93.3%)	66.0-99.6	0 (0%)	0-95.0
E-CAD	8 (66.6%)	35.4-89.0	2 (10%)	1.80-33.1	1 (6.66%)	0.4-34.0	0 (0%)	0-95.0
CD56	6 (50%)	22.2-78.0	1 (5%)	0.30-27.0	1 (6.66%)	0.4-34.0	0 (0%)	0-95.0

FA follicular adenoma, *CG* colloid goitre, *PC* papillary thyroid cancer, *FC* follicular cancer, *CI* confidence interval of a proportion

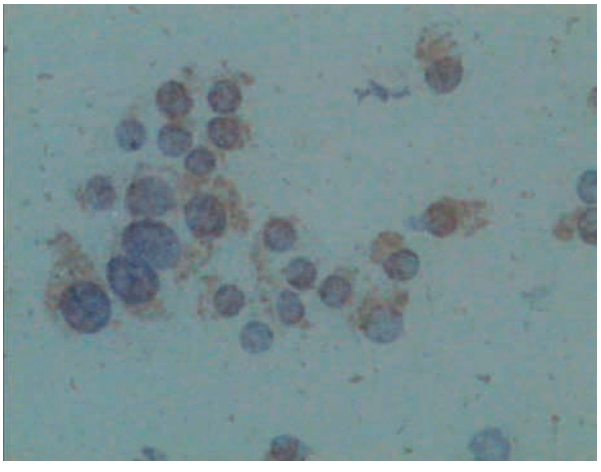

Fig. 1. Cytoplasmic expression of E-cadherin in a group of thyroid epithelial cells. Immunoperoxidase, anti-E-cadherin, original magnification 400x.

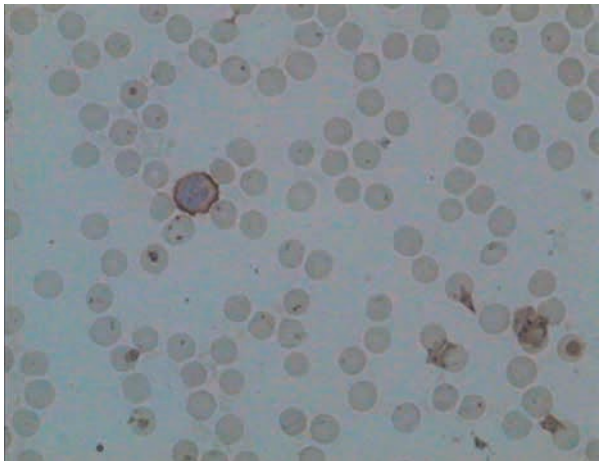

Fig. 2. Intense membranous expression of CD56 in a single epithelial cell despite the rich presence of red blood cells in the clearly suboptimal smear. Immunoperoxidase, anti-CD56, original magnification 400x.

ORIGINAL ARTICLE

Morphological and Immunohistochemical Characteristics of Surgically Removed Paediatric Renal Tumours in Latvia (1997–2010)

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Summary
Introduction. Paediatric renal tumours represent 7% of all childhood malignancies. The variable appearances of the tumours and their rarity make them especially challenging group of lesions for the paediatric pathologist. In Latvia diagnostics and treatment of childhood malignancies is concentrated in Children's Clinical University Hospital. Microscopic evaluation of them is realised in Pathology office of this hospital.
Aim of the study is to analyze morphologic spectrum of children kidney tumours in Latvia and to characterise them from modern positions with wide range of immunohistochemical markers using morphological material of Pathology bureau of Children's Clinical University Hospital.
Materials and methods. We have analyzed surgically removed primary renal tumours in Children Clinical University Hospital from the year 1997 till 2010. Samples were fixed in 10% formalin fluid, imbedded in paraffin and haematoxylin-eosin stained slides were re-examined. Immunohistochemical re-investigation was made in 65.91% of cases. For differential diagnostic purposes were used antibodies for the detection of bcl-2, CD34, EMA, actin, desmin, vimentin, CKAE1/AE3, CK7, Ki67, LCA, WT1, CD99, NSE, chromogranin, synaptophyzin, S100, myoglobin, miogenin, MyoD1 (DakoCytomation) and INI1 protein (Santa Cruz Biotechnology).
Results. During the revised period there were diagnosed 44 renal tumours. Accordingly of morphological examination data neoplasms were divided: 1) nephroblastoma – 75%, 2) clear cell sarcoma – 2.27%, 3) rhabdoid tumour – 4.55%, 4) angiomyolipoma – 4.55%, 5) embrional rhabdomyosarcoma – 2.27%, 6) mesoblastic nephroma – 4.55%, 7) multicystic nephroma – 4.55%, 8) angiosarcoma – 2.27%. Immunohistochemical investigation specify the diagnosis in 4.55% of patients. In case of embryonal rhabdomyosarcoma we found that immunophenotype is more typical for cellular mesoblastic nephroma (infantile renal fibrosarcoma) but one neuroblastoma was misdiagnosed as nephroblastoma.
Conclusions. 1. The most common childhood renal tumours in Latvia like in the world is nephroblastoma. 2. In Latvia there are diagnosed such rare pediatric tumours as clear cell sarcoma and rhabdoid tumour; amount of first one is like in the world, but rhabdoid tumour was more common in Latvia then in the world. 3. No cases of renal cell carcinoma was diagnosed under age of 18 what differs from the data of the world. 4. The results of our investigations proved that immunohistochemical analytic schemes for blastemal type nephroblastoma and mesenchymal kidney tumours in our hospital should be extend but for the precise diagnosis of rhabdoid tumour of kidney would be recommended cooperation between laboratories of several countries for the detection of INI1 protein expression.
Keywords: Paediatric renal tumours in Latvia, immunohistochemistry
Abbreviations: WT – Wilms' tumour, CCSK - clear cell sarcoma of the kidney, RTK - rhabdoid tumour of kidney, MN - mesoblastic nephroma, IFS - infantile fibrosarcoma, CMN - cellular mesoblastic nephroma WT1 – Wilms' tumour gene marker, CKAE1/AE3 - common cytokeratin EMA - epithelial membrane antigen, LCA - leukocyte common antigen, NSE - neuron-specific enolase, H&E - haematoxylin- eosin, PNET – primitive neuroectodermal tumour, MA - metanephric adenoma.

INTRODUCTION

Paediatric renal tumours represent 7% of all tumours in first 15 years of life. Wilms' tumour or nephroblastoma is the most common (85% of cases), followed by renal cell carcinomas (3-5%), mesoblastic nephroma (3%), clear cell sarcoma of the kidney, rhabdoid tumour of the kidney (2%) and miscellaneous rare tumours (2%) [6]. Accurate histological diagnosis and staging of these tumours are critical because their treatment and prognosis are very different. During the past 40 years cooperative groups targeting paediatric renal tumours have been remarkably successful. They have enabled the development of accurate diagnostic criteria, stage and histology-based therapeutic stratifications, and appropriate surgical techniques. In relation to 5 year survival of patients there is a dramatic improvement in prognosis in WT case – from 8% at the beginning of 20th century, to approximately 50% in 1960 and greater than 90% in 2000 [13]. Equally 5 year survival of patients with CCSK has increased from only 20% up to 70% due to the addition doxorubicin to chemotherapeutic protocols [3]. At the same time outcome of RTK is typical dismal, as over 80% of patients will die of tumour within 2 years of diagnosis

[18]. Mesoblastic nephroma in general is benign tumour that classical variant histological is identical to the fibromatosis with a survival rate 100%. The cellular variant is associated with the worse prognosis as a survival rate of 5 years is 85% [1]. Histological cellular variant is identical to the infantile fibrosarcoma and shows translocation t(12; 15)(p13;q25) which leads to the ETV6-NTRK3 gene fusion. Identical translocation has also been reported in fibrosarcoma suggesting that the cellular subtype MN would represent an intrarenal IFS [16]. 5% of the patients with mesoblastic cellular nephroma have recurrence usually within the first year after the nephrectomy and only few cases had metastases [1]. It is believed that completely excised MN is associated with an excellent prognosis. Effectiveness of chemotherapy in CMN case is discussible as not all of them are chemosensitive. Candidates to adjuvant chemotherapy are patients older than 3 month with the cellular variant of tumour, neoplastic tissues in surgical margins, rupture of tumour during resection or vascular microinvasions [1].

AIM TO THE STUDY

Aim of the study is to analyze morphologic spectrum of children kidney tumours in Latvia and to characterise them from modern positions with wide range of immunohistochemical markers using morphological material of Pathology bureau of Children's Clinical University Hospital.

MATERIALS AND METHODS

We have analyzed all surgically removed primary renal tumours cases in Children Clinical University Hospital from 1997 till 2010. In this study we have not included surgical material and biopsies from kidneys due to others reasons: e.g., secondary tumours of kidney or due to retroperitoneal neoplasm like neuroblastoma with invasion into kidney. Primary morphological examination data and tissue material of diagnosed tumours were retrieved from the archive of the Pathology bureau of Children Clinical University Hospital. All tumour tissues were fixed in 10% formalin fluid, imbedded in paraffin, stained with haematoxylin–eosin and re-tested. Complete immunohistochemical investigation was provided in 65.91 % of cases. In all analysed cases were detected WT1, vimentin, CKAE1/AE3, EMA, Ki67 antigen (all antibodies DakoCytomation, Denmark). In addition we have used antibodies for the detection of bcl-2, actin, desmin, CK7, LCA, CD99, NSE, chromogranin, synaptophyzin, S100, myoglobin, miogenin, MyoD1, CD34 (DakoCytomation, Denmark) and INI1 protein (Santa Cruz Biotechnology, USA). Proliferation activity of tumours was assessed counting Ki67 positive cells by magnification x400 and expressed as percentage. Obtained data were analyzed mathematically using computer program Microsoft Excel. Descriptive statistics was used. There is no ethical conflict in this report; the principles outlined in the Declaration of Helsinki were followed by authors.

RESULTS

Due to the clinically diagnosed primary renal tumours 45 nephrectomies and 3 partial kidney resection were done in years 1997-2010. The tumour diagnosis was confirmed in 91.67% of cases (n=44), but in 8.33% (n=4) pathologist findings did not meet the clinical diagnosis of tumour. 6.25% (n=3) of cases the clinical diagnosis was primary renal tumour (lymphoma, renal cell cancer) but histologically was found xanthogranulomatous pyelonephritis. In 2.08% (n=1) pathological process morphologically was not found. The amount of analyzed paediatric renal tumours during overviewed period 1997-2010 is showed in Fig.1.

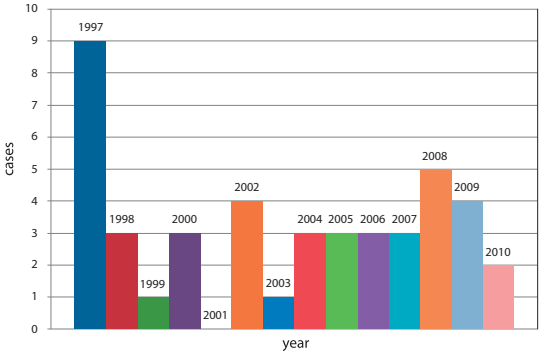

Fig. 1. Number of Primary Paediatric Renal Tumours in Children's Clinical University Hospital (1997_2010), Riga, Latvia.

Our morphological examinations had proved such different subtypes of renal tumours:
1) nephroblastoma – 75% (n=33), 2) clear cell sarcoma – 2.27% (n=1), 3) rhabdoid tumour - 4, 55% (n=2), 4) angiomyolipoma – 4, 55% (n=2), 5) embrional rhabdomyosarcoma – 2,27% (n=1), 6) mesoblastic nephroma – 4,55% (n=2), 7) multicystic nephroma – 4,55% (n=2), 8) angiosarcoma- 2, 27% (n=1).
After modern immunohistochemical analysis one case of nephroblastoma was interpreted as neuroblastoma. Histologically the tumour cells were small and regular, with round, deeply staining nuclei and little cytoplasm. Few Homer Wright rosettes were present. Immunohistochemistry showed complete lack of WT1expression, the tumour had no EMA and CKAE1/AE3 positive epithelial structures but reaction with neuronal markers as NSE, synatophysin and chromogranin showed strong immunoexpression. Reactions for other childhood small round cell tumours markers as LCA, desmin, vimentin, aktin, myoglobin, myogenin and CD99 were negative. Ki67 labelling index was 40%. In one other nephroblastoma case re-investigation was encountered differential diagnostic difficulty. Histological diagnosis in the morphological investigation was nephroblastoma mixed (epithelial blastemal) type. Tumour was well circumscribed but not encapsulated, the entire tumour was composed of primitive, compactly arranged small acinar epithelial structures, necrosis and vascular invasion were not found. The acini were variably arranged, dense to loosely

scattered in acellular stroma. Immunohistochemically was found diffuse membranous CKAE1/AE3 positivity in all tumour cells but EMA and vimentin were negative. Nuclear reaction with WT1 was positive in all tumour cells (Fig.2), CK7 was negative in tumour cells but positive in normal tubular epithelium showed. Ki67 expression was patchy. The average size of Ki67 was 18, 44%. Histological and immunohistochemical picture of this case is more characteristic for metanephric adenoma [12] but finding of proliferation index excludes diagnosis of adenoma therefore was decided to establish a diagnosis nephroblastoma epithelial type (pattern like metanephric adenoma).

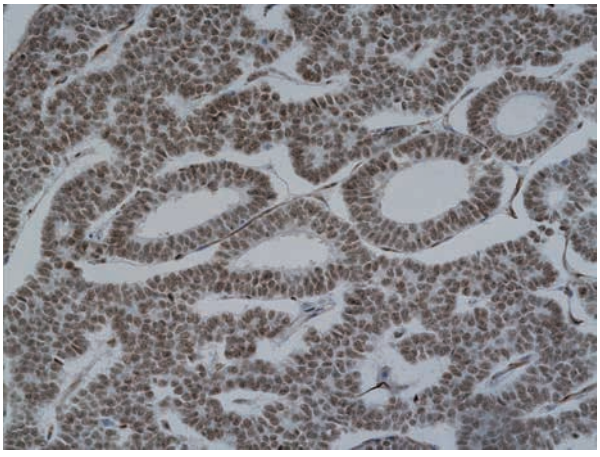

Fig. 2. Wilms' tumour epithelial (pattern like metanephric adenoma) WT1 immunoexpression in epithelial cells nuclei. Magnification x200.

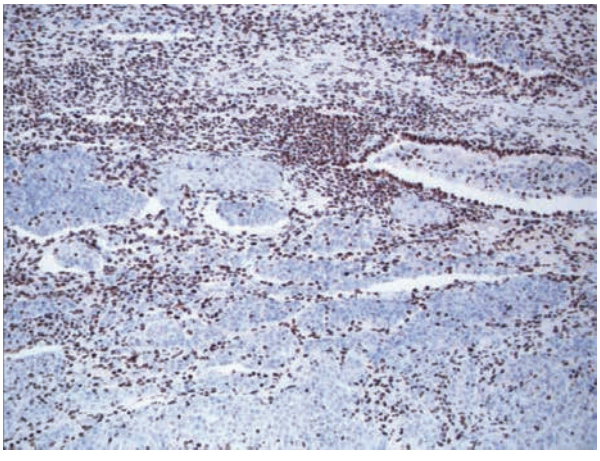

Fig. 3. Rhabdoid tumour of kidney – loss of INI1 protein expression in tumour cell nuclei. Magnification x200.

From 32 nephroblastoma patients 16 were male and 16 were female (M/F =1). Patients age was from 8 months till 17 years 11 months (average 64.15 months, standard deviation 61.31, mode 36) but 62.5% (n=20) were patients till the age of 4 years. The morphologic types of WT were: blastemal - 34.37% (n=11), epithelial - 31.25% (n=10), mixed (epithelial blastemal) - 12.5% (n=4), mesenchymal - 12.5% (n=4), regressive - 6.25% (n=2), 3.12% (n=1) was found cystic nephroblastoma. Only in one case or 3.12% of the all analyzed samples diffuse anaplasia (unfavourable histology) was found. Tumour belonged to the blastemal type nephroblastoma group. Patient was 8 months old boy, 4 months after the first surgery developed tumour recurrence. Nine (90%) of the ten epithelial type nephroblastoma cases showed tubular differentiation, in one case (10%) could be seen glomerular differentiation. In one of the tubular differentiation case has been above described pattern like metanephric adenoma.

During the analysis of other non WT cases immunohistochemistry was made in six cases of them. In two cases the primary morphological investigation approved diagnosis of rhabdoid tumour. In one of cases the tumour cells typically showed the cytological triad of vesicular chromatin, prominent cherry-red nucleoli and hyaline pink cytoplasm inclusions. In the second case cytoplasm inclusions were not prominent and diagnostic difficulties were caused by extensive tumour necrosis too. Immunohistochemical analysis in both cases showed strong vimentin positivity and focal positivity of EMA and CKAE1/AE3. Reaction for the detection of WT1, bcl-2, muscle specific actin, S100, CD99, NSE, LCA, desmin, synaptophysin, and CD34 mainly were negative in tumour cells. In both cases there was detected INI1 protein expression in tumour cells and was found extensive loss of nuclear staining in tumour cells, whilst the nuclei of adjacent normal cells retain their pattern (Fig.3) corresponding to the described immunoprofile of rhabdoid tumour [2].

During reviewed period there was also diagnosed such rare primary renal tumour as clear cell sarcoma. Histologically tumour was composed of spindled cells, which were separated by extracellular myxoid material that mimics clear cytoplasm. Immunohistochemical investigation showed bcl-2 and vimentin positivity (Fig.4), reaction for the detection of WT1, CD34, EMA, muscle specific actin, desmin, CKAE1/AE3, LCA was negative (Fig.5). That pattern is consistent with literature data about immunoprofile of clear cell sarcoma of kidney [17].

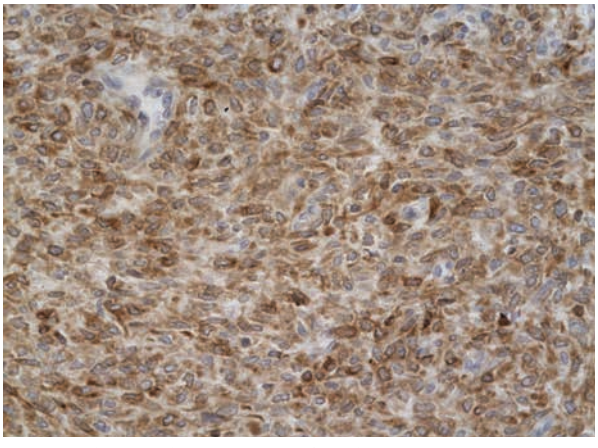

Fig. 4. Clear cell sarcoma of kidney. Bcl-2 positivity in the cytoplasm of tumour cells. Magnification x200.

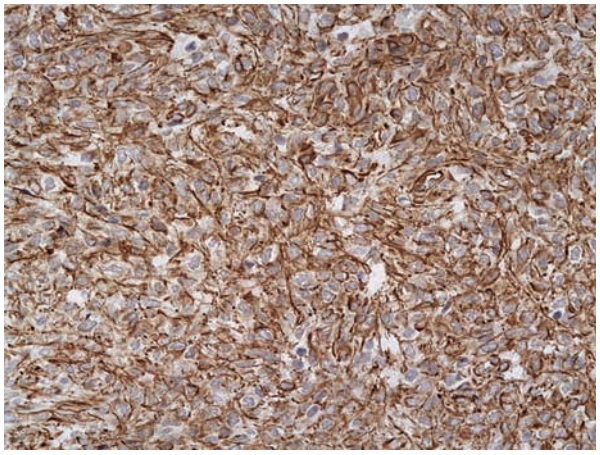

Fig. 5. Clear cell sarcoma of kidney. Nuclear WT1 negativity, non-specific reaction in the cytoplasm of tumour cells Magnification x200.

By immunohistochemical re-investigation diagnosis of embryonal rhabdomyosarcoma was changed to cellular mesoblastic nephroma. Patient was 25 days old girl with congenital tumour of kidney. Tumour was composed of plump spindle cells with histologically features of myofibroblasts that infiltrate adjacent tissue. Some necroses were present. Complete immunohistochemistry showed: vimentin positivity, reaction for muscular markers as MyoD1, muscle specific actin, desmin, myogenin was negative. Others markers as WT1, EMA, CKAE1/AE3, CD34, CD31, LCA were negative, reaction with bcl-2 showed rare cells positivity. Ki67 labelling index was 44.44%. Accordingly to clinical data, histological picture and complete immunohistochemistry there were decided to specify a diagnosis as cellular mesoblastic nephroma (infantile renal fibrosarcoma).

Both cases of angiomyolipoma were analyzed immunohistochemically too and they had typical histological pattern. Tumours were composed of adipose tissue, spindle and epitheloid smooth muscle cells and abnormal thick-walled blood vessels [8]. Immunohistochemistry showed desmin and muscle specific actin positivity, epithelial markers as EMA and CKAE1/AE3 were negative, CD34 was positive in vascular structures corresponding to the picture of angiomiolipoma. Ki67 labelling index was 4% in first case and less than 1% in second case.

Both cases of cystic nephroma were revised only in H&E specimens as tumours had characteristic histological pattern and there were lack of immunohistochemical markers. Histologically tumours were composed entirely of cysts with delicate septa, cysts were lined with cuboidal epithelium or lack lining epithelium, and septa were variably cellular and contained undifferentiated and differentiated mesenchyme. Blastema and nephroblastomatous epithelial elements were no found.

Summarizing the data of all analyzed tumours, it can be seen, that accordingly of morphological investigation as well as immunohistochemical analysis data in general between renal tumours of kids in Latvia mainly is nephroblastoma (75% after histological investigation and 72.73% after immunohistochemistry). In our research also rare paediatric primary renal tumours have been approved as rhabdoid tumour, clear cell sarcoma and angiomyolipoma. In two cases (4.54%) immunohistochemical re-investigation clarified the diagnosis. Diagnosis of embrional rhabdomiosarcoma was changed to cellular mesoblastic nephroma and one of nephroblastoma diagnosis was changed to neuroblastoma. Primary and re-investigation data are showed in Fig.6.

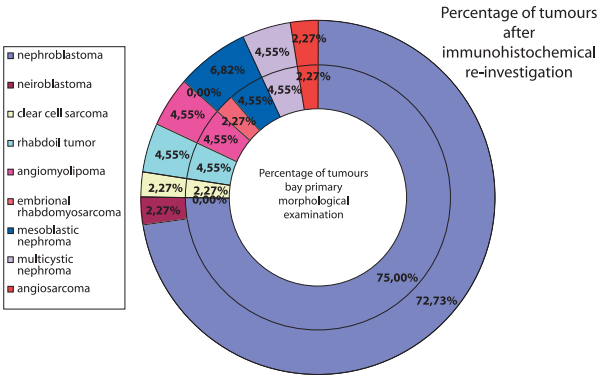

Fig. 6. The amount of renal tumours in percentage accordingly primary morphological and immunohistochemical re-investigation data.

DISCUSSION

Analyzing the results of current research everyone should take into account the fact that all investigated renal children tumours are rare in Latvia and the total number of tumours is small. In our investigation we met the situations that tumour's tissue quality not always allowed the full imunohistochemical investigation but the review of H&E slides of all tissue material showed that there was insufficient justification to change an existing morphological diagnosis and exclude cases from a primary renal tumours group. Therefore, some mesoblastic nephroma cases as well as angiosarcoma were included in the total spectrum of tumours. Despite the problems we believe that it is absolutely necessary to research diagnosed material to gather experience and improve the diagnostic quality as well as present paediatric renal tumour spectrum of Latvia. Our study showed that in Children's Clinical University Hospital like in the world between renal tumours of kids mainly was nephroblastoma but spectrum of the other morphological type was enough broad and are diagnosed such rare primary renal neoplasms as rhabdoid tumour and clear cell sarcoma. In comparison Lithuanian colleagues in Vilnius University Children's Hospital from 1997 till 2008 were investigated 30 primary renal tumours. 93.7% of them were WT but 6.3% renal cell carcinoma [11]. Opposite in our hospital

there was not detected malignant renal epithelial neoplasm in children and this diagnosis was not established in our immunohistochemical investigation too. Our re-investigation of all surgically removed renal tumours diagnosis has clarified in 4.54% of cases. Results of immunohistochemical analysis revealed some questions which should be addressed to practitioners. Each must take into consideration that usually WT is a tumour with classic histological picture composed of variable mixtures of blastema, epithelium and stroma, but there are cases when in some tumours only two and occasionally only one component is present. Diagnostic problems usually provide differentiation between blastemal type WT and other "small round cell tumour" especially neuroblastoma, PNET, renal lymphoma as well as CCSK and RTK [15]. In our study complete immunohistochemical investigation showed immunoprofile of neuroblastoma in one of blastemal type WT case. This problem must be solved with a broad immunohistochemical investigation scheme including detection of WT1, neuroendocrine, epithelial markers, LCA and CD99 markers [15; 7]. It should be noted that nephroblastoma is characterized by vimentin positivity in blastemal cells while the mature epithelial structures showed positivity of epithelial markers [7].Positive staining of Wilms' tumour gene marker in blastemal cells and early epithelial structures is present around 70% of WT cases [5]. Morphological differentiation between metanephric adenoma and WT may be difficult too. MA is an uncommon renal epithelial neoplasm of primitive appearance that belongs to the spectrum of nephrogenic rest- derived tumours, which also include WT, metanephric adenofibroma and metanephric stromal tumour. Although WT is thought to represent the malignant end of this spectrum, the other metanephric neoplasms are generally regarded as benign. Immunohistochemistry of MA often shows diffuse nuclear positivity with WT1 and variable expression of epithelial markers. In our case critical was detection of Ki67 labelling index because in described adenomas cases it was low. It may be less than 1% and sometimes achieves the 5% level [9, 12] therefore the diagnosis of adenoma was not confirmed. Correct diagnosis of RTK really is difficult question in paediatric pathologist practice too. Although in most of cases diagnosis could be made correctly on the basis of careful interpretation of light microscopic details but there is sufficient opportunity to make mistakes. Tumours originally diagnosed as rhabdoid tumour of kidney often were found to be other renal tumour and vice versa in a lot of studies in other countries too [19; 20]. A wide range of renal neoplasms mimicking RTK was described. It consist of a diverse group of neoplasms, including anaplastic WT, congenital mesoblastic nephroma, renal cell carcinoma, rhabdomyosarcoma, malignant neuroepithelial tumours and lymphoma [20].Molecular hallmark of rhabdoid tumour is biallelic inactivation of the *hsNF5/INI1* tumour suppressor gene. This leads to loss of INI1 protein expression, which may serve as a useful immunohistochemical marker in infants and young children. [2;14]. In one of our cases was not a classic picture of tumour therefore INI1 protein expression was immunohistochemicaly investigated in both cases and diagnosis were verified in Our Lady's Children's Hospital, Dublin (Ireland). Currently in Latvia detection of INI1 protein is too expensive as RTK is rare tumour and the period between our two RTK cases was five years. Therefore we recommend cooperation between laboratories of some countries to detection of INI1 protein expression to specify diagnosis.

The case of diagnosed rhabdomyosarcoma was also carefully immunohistochemicaly investigated but it is really rare one. Grignon et al. reviewed the literature and found only eight convincing cases [10]. Most renal tumours with appearances suggesting rhabdomyosarcoma are something another [8]. In our case, detailed immunohistochemical investigation findings with vimentin positivity, above mentioned muscular markers, WT1 and bcl-2 negativity is more appropriate for cellular mesoblastic nephroma [4]. Proliferative activity was high. Ki67 level was 44, 44% in comparison with that one described in literature 20% -15% [21]. Therefore pathologist's choice to make diagnosis of malignant tumour during morphological investigation is understandable but next one could be recommended critically to evaluate diagnosis of primary renal rhabdomyosarcoma taking into account differential diagnosis of cellular mesoblastic nephroma especially since here is the question about adequate chemotherapy.

CONCLUSIONS

1. The most common childhood renal tumours in Latvia like in the world is nephroblastoma.
2. In Latvia there are diagnosed such rare pediatric tumours as clear cell sarcoma and rhabdoid tumour; amount of first one is like in the world, but rhabdoid tumour was more common in Latvia then in the world.
3. No cases of renal cell carcinoma was diagnosed under age of 18 what differs from the data of the world.
4. The results of our investigations proved that immunohistochemical analytic schemes for blastemal type nephroblastoma and mesenchymal kidney tumours in our hospital should be extend but for the precise diagnosis of rhabdoid tumour of kidney would be recommended cooperation between laboratories of several countries for the detection of INI1 protein expression.

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Conflict of interest: None

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ORIGINAL ARTICLE

Magnetic Resonance Spectroscopy for Evaluation of Brain Glioma Extent

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Summary
Introduction. Based on the long observations, gliomas recur predominantly within 1 centimeter of the resection margin. This is mainly due to the fact that at the time of surgical removal, cells from the core of the tumor have already invaded the peritumoral area that appears unchanged on structural magnetic resonance (MR) images. MR spectroscopy provides a noninvasive assessment of tumor metabolism with which the concentration of N-acetylaspartate (NAA), choline (Cho), creatine (Cr), lactate and lipid (LL), myo-inositol (MI) can be determined.
Aim of the study. The purpose of this study was to evaluate the neurometabolites ratios as an indicator of the glial brain tumors infiltration into the surrounding tissue.
Materials and methods. MR spectroscopy was performed in 54 patients with histologically confirmed brain glioma. Patients were divided into two groups based on the time of MR examination performed: before and after therapy. Regions of interest (ROIs) were placed in the tumor centre, the perifocal edema zone, the distant and contralateral normal-appearing white matter. Mean ratios of NAA/Cr, Cho/Cr, MI/Cr, LL/Cr were calculated in each ROIs.
Results. In the both groups the Cho/Cr ratios were significantly elevated in the tumor and the edema zone; the NAA/Cr ratios were significantly reduced in the tumor; the LL/Cr ratios were significantly elevated in the tumor and the edema compared with the contralateral hemisphere.
In the second group the Cho/Cr and LL/Cr ratios in the edema zone was significantly lower than in the tumor and significantly higher than in the distant white matter; the Cho/Cr, MI/Cr and LL/Cr ratios in the tumor were significantly higher than in the distant white matter.
Conclusions. The analysis of spatial distribution of metabolites ratios by MR spectroscopy helps to discriminate among tumor and normal tissues, offering information not available with structural MR. LL/Cr ratios may have the superior implications in the detecting of glial tumors extent. Metabolic changes in the peritumoral area demonstrated by MR spectroscopy can assist in choosing a biopsy target for brain gliomas and in planning of surgical removal or radiation therapy.
Key words: magnetic resonance spectroscopy, brain glioma, peritumoral area.

INTRODUCTION

Based on the long observations, gliomas recur predominantly within 1 centimeter of the resection margin even after apparently complete gross resection. This is mainly due to the fact that at the time of surgical removal, cells from the core of the tumor have already invaded normal brain tissue. Single invasive cells can be detected histologically even several centimeters away from the bulk of the lesion (6, 8). With the structural MR the location, size, shape, and structure of glioma can be detected but not the extent of tumor infiltration in the white matter (9, 12). MR spectroscopy provides a noninvasive, quantitative assessment of tumor metabolism with which the concentration of neurometabolites can be determined (1, 5, 9, 13, 16). NAA is a marker of neuronal integrity (2, 9, 10, 15, 16). Cho is a marker of cell membranes turnover (9, 15, 16, 21). Cr reflects information on energy metabolism (9, 21). The Cr peak remains relatively stable under most conditions (11). LL peaks are not observed in a normal brain spectrum. Lactate is an end-product of anaerobic glycolysis (13). Lipids are the products of brain destruction (10). MI is a marker of astrocytes, it acts as an osmolyte (3, 12, 16).

Previously reported MR spectroscopy findings in brain tumors comprised a decrease in NAA, mildly decrease in Cr, an increase in Cho and presence of LL peaks (2, 10, 11, 13, 15). MI is higher in low-grade gliomas and lower in high-grade gliomas compared with control subjects (3, 10). Many authors have reported the correlation of metabolite ratios with histopathological grading of brain gliomas (5, 11, 15, 22). Various studies have shown that MR spectroscopy is valuable for differentiating tumor from normal tissue (5, 9, 13). In this study we analysed the spatial metabolic distribution within gliomas, perifocal edema and surrounding normal tissues by Cho, NAA, MI, LL ratios against Cr, which is a constant metabolite that is not significantly altered by infiltrative diseases (9, 21).

AIM OF THE STUDY

The purpose of this study was to evaluate the neurometabolites ratios as an indicator of the glial brain tumors infiltration into the surrounding tissue.

MATERIALS AND METHODS

The local institutional review board of the Riga East University Hospital and the Ethics committee of Riga Stradins University approved this study.

Study participants

Between August 2009 and July 2011, MR spectroscopy was performed in 54 patients (23 men, 31 women) with histologically confirmed brain glioma. Histologic confirmation of the tumors was achieved by gross total or partial resection. Mean age of all patients was 44 years (range, 14-73 years). Inclusion criteria for patients were histologic confirmation of brain glioma. Therefore patients without histologic confirmation were excluded from this investigation. Histological type was classified according to the World Health Organization brain tumor classification (14). The studied neoplasms consisted of 28 glioblastomas, 9 anaplastic oligoastrocytomas, 9 anaplastic astrocytomas, 2 oligoastrocytomas, 2 astrocytomas, 1 anaplastic oligodendroglioma, 1 oligodendroglioma, 1 anaplastic pleomorphic xanthoastrocytoma, 1 gliosarcoma.

Patients were divided into two groups based on the time of MR examination performed: before and after surgery or combination therapy. The first group comprised 9 patients with primary diagnosed glioma who underwent MR examination before treatment. The second group comprised 45 patients with residual/recurrent glioma who underwent MR examination after previous treatments.

MR imaging

All examinations were performed with a 1,5T MR unit (GE Signa EXCITE MR) including structural MR sequences (T2-weighted, FLAIR, diffusion-weighted, T1-weighted imaging before and after intravenous gadolinium-based contrast media administration) and MR spectroscopy. Our standard routine clinical protocol involved the use of eight-channel head coil. MR spectroscopy was performed with 2D multivoxel chemical shift imaging - (8ch)PROBE-2DSI PRESS 144TE. Technical parameters of MR spectroscopy were: echo time, 144ms; repetition time, 1000ms; NEX, 1; acquisition time, 4 minutes, 20 seconds. MR spectroscopy images were acquired before administering the contrast agent. The volumes of interest were chosen to include as much as possible of the tumor, peritumoral area and normal contralateral brain regions, and to exclude subcutaneous fat and sinuses in accordance with previous guidance (5).

MR data evaluation

All MR images were transferred to a workstation (MR GELS) for analysis. MR spectra were analyzed using the Functool software. The axial T2-weighted images or FLAIR images were used to place defined ROIs in spectroscopic matrix in the tumor centre, the perifocal edema, the normal-appearing white matter in distance 5-10 millimetres from the altered signal intensity area (distant normal-appearing white matter) and in the normal-appearing white matter of the contralateral hemisphere (contralateral normal-appearing white matter). ROIs were defined on the basis of following imaging features adopted in the main principles of previous studies (2, 5): tumor centre, a region containing a well defined solid portion, contrast enhancement, and abnormal signal intensity on T2-weighted and FLAIR images; perifocal edema, a region containing no enhancement and higher signal intensity on T2-weighted and FLAIR images; normal-appearing white matter, an area containing normal signal intensity with no enhancement on all structural MR sequences (Fig.1.A, B). Mean metabolite ratios of NAA/Cr, Cho/Cr, MI/Cr, LL/Cr were calculated in each ROIs.

Statistical analysis

Statistical analyses were realized using the SPSS version 14.0 software. The mean and standard deviations (SD) of all MR spectroscopy ratios were calculated. Variables in each group were examined for their compliance with normal distribution by means of Kolmogorov-Smirnov test. In the first group all variables demonstrated normal distribution ($p>0.05$), therefore the Student's t-test for paired samples was used. In the second group several variables did not conform to normal distribution ($p<0.05$), therefore the non-parametric Wilcoxon signed-rank test was used to compare paired samples. P values less than 0.05 were considered to indicate a statistically significant difference.

RESULTS

First group - primary diagnosed glioma

The measurements of metabolite ratios according to different ROIs are summarized in Table 1. Compared with the contralateral hemisphere, the mean values of the Cho/Cr ratios were significantly elevated in the tumor ($p=0.035$), the edema zone ($p=0.025$) and the distant normal-appearing white matter ($p=0.014$); the mean values of the NAA/Cr ratios were significantly reduced in the tumor ($p=0.041$); the mean values of the LL/Cr ratios were significantly elevated in the tumor ($p=0.002$) and the edema ($p=0.01$). No significant differences were noted in MI/Cr ratios between different ROIs. Mean metabolites ratios in the different ROIs are shown in the line graph in Fig.2.

Second group - residual/recurrent glioma

The measurements of metabolite ratios according to different ROIs are summarized in Table 2. Compared with the contralateral hemisphere, the mean values of the Cho/Cr ratios were significantly elevated in the tumor ($p<0.001$), the edema ($p=0.011$); the mean values of the NAA/Cr ratios were significantly reduced in the tumor ($p=0.002$), the edema ($p=0.002$), the distant normal-appearing white matter ($p=0.003$); the mean values of the MI/Cr ratios were significantly elevated in the tumor ($p=0.019$); the mean values of the LL/Cr ratios were significantly elevated in the tumor ($p<0.001$), the edema ($p<0.001$) and the distant normal-appearing white matter ($p<0.001$).

Mean Cho/Cr ratio in the edema was significantly lower than in the tumor ($p=0.029$) and significantly higher than in the distant normal-appearing white matter ($p=0.013$). In the same pattern, mean LL/Cr ratio in the edema was significantly lower than in the tumor ($p=0.04$) and significantly higher than in the distant normal-appearing white matter ($p<0.001$).

Compared with the distant normal-appearing white matter, mean values of the Cho/Cr, MI/Cr and LL/Cr ratios were significantly elevated in the tumor ($p<0.001$). Mean metabolites ratios in the different ROIs are shown in the line graph in Fig.3.

DISCUSSION

The depiction of true margins of gliomas can be quite difficult on structural MR imaging, and this information is critical for the correct therapeutic management (5). This study aimed to evaluate the usefulness of MR spectroscopy in differentiating gliomas from edema and normal tissue and which parameter was most effective in enhancing such differentiation. Our study of the second group revealed continuous increase of LL/Cr ratios from surrounding tissue of the tumor toward the centre. In the first group we also observed similar tendency, only mild decline of LL/Cr ratios was detected in the tumor centre. Such difference could be related with effects of previous treatments in the second group. LL is directly related to necrosis (11, 23). Developing necrosis is likely characterized by progressive increase of lipid, due to cell breakdown (5, 10). In previous histopathological studies of biopsy and autopsy specimens were revealed that morphological changes induced by radiation and chemotherapy are characterized by cell necrosis (7). The implication of LL/Cr ratios in the determination of tumor boundaries is described also in the previous reports (18).

Our analysis of MR spectroscopy findings in comparison with the contralateral normal-appearing white matter in the both groups showed that the mean values of the NAA/Cr ratios were significantly reduced in the tumor. Histologically it could be explained with a decreased number or an absence of neurons in the tumor (11). In the second group we also revealed significantly reduced NAA/Cr ratios in the peritumoral area. It could be indicative of some invasive cells outside of tumor core – in the peritumoral area that appears unchanged on structural MR images. Previous study revealed that tumors actively induce neuronal death at the growing tumor margins (20).

Significant differences in the Cho/Cr ratios between the tumor centre and the normal-appearing contralateral brain parenchyma were obtained on both groups, which was in good agreement with previous report (22). In both groups we observed continuous increase of Cho/Cr ratios from surrounding tissues toward the tumor. Cho is component in cell membrane and suggests cell turnover (19). The elevation in the Cho/Cr ratios reveals an increase of cell turnover, which becomes more pronounced toward the tumor centre.

One of the major findings in the present study was the metabolite changes in the distant normal-appearing white matter. As observed by some authors, recurrent gliomas arise from tumor cells present in this region around the resection margins even after apparently complete gross resection (17). Analyzing the distant and the contralateral normal-appearing white matter we found a significant difference of Cho/Cr ratios in the first group and NAA/Cr and LL/Cr ratios in the second group. These results could indicate of some invasive tumor cells in the surrounding tissues. Structural MR imaging reflects only part of the tumor which is often more extensive and infiltrating than the altered signal intensity alone. MR spectroscopy gives an overview of the tumor as a whole (1). Increase in the LL/Cr ratio was the most significant MR spectroscopic parameter in the evaluation of tumor cells invasion in the second group of patients. In the first group there was a similar trend of an increase in LL/Cr ratio in distant normal-appearing white matter, but we could not find any statistically significant relation between the distant and the contralateral area.

Histologically regions of altered signal intensity outside the enhancing margins of high-grade gliomas comprise a combination of vasogenic edema and infiltrating tumor cells (5). Compared with the tumor centre we found significantly reduced Cho/Cr and LL/Cr ratios in the perifocal edema zone in the second group. In the first group of untreated patients we did not find a statistically significant difference probably due to greater amount of vasogenic edema. Some authors suggested that the presence of water probably dilutes the typical tumor spectra (5).

In our study the MI/Cr ratios showed the most inferior diagnostic performance for evaluation of peritumoral area. We found only significant differences in MI/Cr ratios between the tumor centre and the normal-appearing white matter in the second group. The present study confirms previous findings (4) that MI/Cr ratios of the peritumoral area will not improve the accuracy of diagnosis. Some authors suggested that MI/Cr ratios correlate with the histological grade of cerebral astrocytic tumors (3).

A limitation of this study is the relatively small number of patients in the first group. Although the similar tendencies of metabolite ratios from different brain regions, especially in Cho/Cr and LL/Cr, were obtained. Therefore, further research involving larger patient cohort with primary diagnosed glioma might expand our results.

Our data confirmed that elevation in Cho/Cr and LL/Cr with reduction of NAA/Cr is a reliable indicator of brain glioma. The results of present study demonstrate that MR spectroscopy, especially with LL/Cr measurements, can detect infiltration of glioma cells in the peritumoral areas.

CONCLUSIONS

The analysis of spatial distribution of metabolites ratios by MR spectroscopy helps to discriminate among tumor and normal tissues, offering information not available with structural MR. LL/Cr ratios may have the superior implications in the detecting of glial tumors extent. Metabolic changes in the peritumoral area demonstrated by MR spectroscopy can assist in choosing a biopsy target for brain gliomas and in planning of surgical removal or radiation therapy.

Conflict of interest: None

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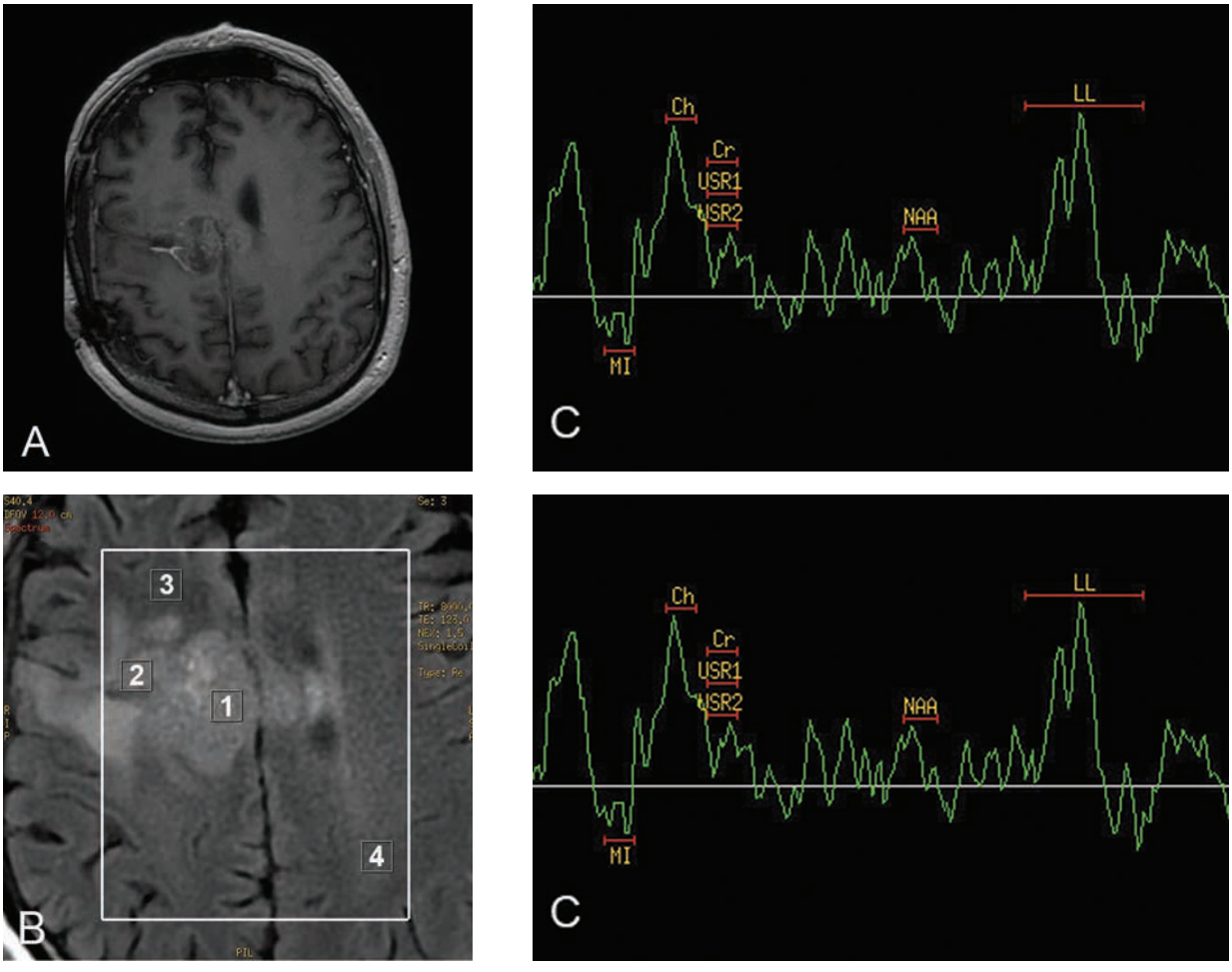

Fig. 1. MR and MR spectroscopy images obtained in 51-year-old man with recurrent glioblastoma. A. Gadolinium-enhanced T1-weighted axial image reveals an inhomogeneously enhancing lesion in the right frontal-parietal lobe extending to the corpus callosum. B. The spectroscopic matrix on axial FLAIR image demonstrates the positioning of ROIs in the tumor centre [1], the perifocal edema [2], the distant [3] and the contralateral normal-appearing white matter [4]. C. Spectrum from the tumor area reveals increased Cho/Cr ratio, decreased NAA/Cr ratio and LL peak. D. Spectrum from normal-appearing contralateral brain parenchyma.

Table 1. Summary measurements (mean±SD) in the first group of the patients with primary diagnosed glioma

Metabolites ratios	Tumor	Perifocal edema	Distant normal-appearing white matter	Contralateral normal-appearing white matter
Cho/Cr	2.37±2.06	1.50±0.82	1.27±0.47	0.69±0.16
NAA/Cr	1.02±0.56	2.19±1.92	1.52±0.76	2.31±1.51
MI/Cr	0.84±0.51	0.90±0.65	0.56±0.32	0.69±0.49
LL/Cr	4.03±1.94	4.38±2.26	2.89±2.34	1.28±0.44

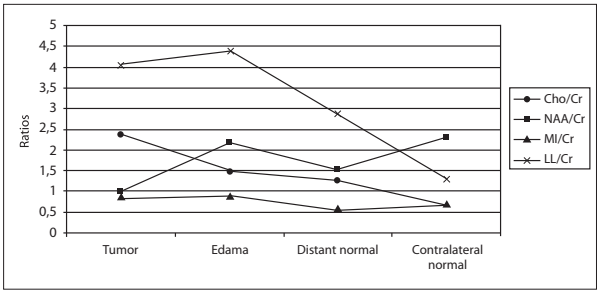

Fig. 2. Mean metabolites ratios in the different ROIs in the first group of patients

Table 2. Summary measurements (mean±SD) in the second group of the patients with residual/ recurrent glioma

Metabolites ratios	Tumor	Perifocal edema	Distant normal-appearing white matter	Contralateral normal-appearing white matter
Cho/Cr	2.39±1.67	1.77±1.55	1.11±0.62	1.05±0,45
NAA/Cr	1.48±1.25	1.59±1.01	1.71±1.04	2.31±1.27
MI/Cr	0.93±0.64	0.94±0.96	0.57±0.46	0.69±1.10
LL/Cr	4.32±2.52	3.48±2.43	1.94±1.14	1.22±0.39

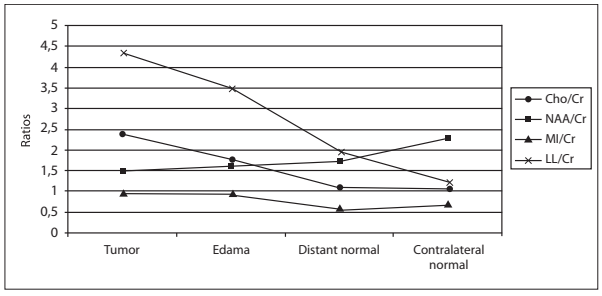

Fig. 3. Mean metabolites ratios in the different ROIs in the second group of patients

ORIGINAL ARTICLE

Endovascular Thrombectomy in Treatment of Patients with Acute Ischemic Stroke – Pauls Stradins Clinical University Hospital Experience

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Summary
Introduction. 15 mlj people suffer from stroke each year (World Health Organization 2005). That is one of the main causes of mortality,dementia and disability. (5;7) Inability after stroke is much more important problem than mortality, since it forms substantial additional costs in the health and social budget , and often isolate patients from the social life.
Aim of the study. The aim of our study is to evaluate the safety and effectiveness of mechanical thrombectomy and cerebrovascular revascularization for patients with acute ischemic stroke.
Materials and methods. This study was retrospective and all included patients (n=37) were hospitalized in Department of Neurology at Pauls Stradins Clinical University hospital with acute ischemic stroke. Patients with carotid artery, middle cerebral artery and basilar artery occlusions were included in the thrombectomy group.
Results. Our results suggest that endovascular revascularization for patients with acute ischemic stroke in case of large artery occlusion is relatively safe and effective. This method is a method of choice for ischemic stroke patients with middle cerebral, basilar artery, and carotid artery occlusion.
Conclusions. Endovascular revascularization is relatively safe and effective method of stroke treatment.
Key words: endovascular revascularization, stroke, thrombectomy.

INTRODUCTION

15 million people suffer from stroke each year (World Health Organization 2005). Stroke is one of the main causes of mortality, dementia and disability (5;7). Disability after stroke is much more important problem than mortality, since it forms substantial additional costs in the budget of health and social care, and often isolate patients from the society. In Latvia yearly from stroke (ischemic and hemorrhagic) die 230/100 000 people aged35 to 74 years, and that is one of the worst rate in Europe(9). The incidence of cerebral infarction in Latvia, taking into account only the number of patients hospitalized in 2004 was up to 284/100 000 inhabitants, which in comparison to European countries is sufficiently high score (6). As one of the possible solution of this problem could be effective and versatile treatment, which is focused on the remove of pathogenic and etiologic moment of disease.

The area surrounding an infarct zone that suffers less severe from ischemia is called the ischemic penumbra. It is considered dysfunctional but not irreversibly damaged tissue, and is potentially salvageable in case if adequate flow is restored. (4) Intravenous (IV) tissue plasminogen activator (tPA) achieves early recanalization in only 30%–50% of patients, with even lower recanalization rates in proximal large vessel occlusions (middle cerebral, basilar artery, and carotid artery) (2;13), besides reocclusion of the vascular segment occurs frequently. (1;3) Endovascular mechanical thrombectomy can restore vascular patency of these vessels between 81% and 94% with good clinical outcome. (8;10-12)

AIM OF THE STUDY

The aim of our study was to evaluate the safety and effectiveness of mechanical thrombectomy and cerebrovascular revascularization for patients with acute ischemic stroke.

MATERIALS AND METHODS

The study was done at the P.Stradins Clinical University Hospital's Department of Neurology during the period from April 2010 to May 2011. The diagnosis of stroke was defined according to WHO definition: as a focal (or at times global) neurological impairment of sudden onset, and lasting more than 24 hours (or leading to death), and of presumed vascular origin. Inclusion criterion for the study was first – ever stroke or recurring cerebral infarction with carotid artery, middle cerebral artery M1 segment or basilar artery occlusion. Patients who were not eligible for intravenous thrombolysis or known to have suboptimal outcome after that, were included if time from symptom onset was less than 8 hours for anterior circulation and less than 24 hours for basilar artery occlusion. Decision to do endovascular thrombectomy was done together by stroke neurologist and interventional radiologist after performing neurological and radiological (CT, CT angiography and CT perfussion) evaluation. From 37 patients treated in

total, there were basilar artery occlusion in 9 (24%), occlusion of internal carotid artery - 2 (5%), tandem occlusion of internal carotid and M1 segment – 8 (22%), occlusion of M1 segment – 18 (49%) patients. NIHSS was evaluated in all patients by neurologist before treatment and at discharge.

RESULTS

Successfull revascularization defined as TICI (thrombosis in cerebral ischemia) scale 2b or 3 was achieved in 92% of treated vessels. There were 2 cases (5%) of distal thrombembolic events during revascularization with corresponding ischemic lesions. There were 4 patients (10,8%) with symptomatic postprocedural intracerebral hemorrhage. Overall mortality was 13,5%, but only 8% due to unsuccesfull revascularization or complications related to treatment. Mean NIHSS rate before treatment was 18,4 (6-36), improved to 7,5 (0-24) at discharge. Before treatment NIHSS rate ≥10 was in 86,5% of patients. Good outcome was considered in patients having NIHSS rate <8 at discharge, which was achieved in 54%.

DISCUSSION

In this retrospective study we demonstrated our experience about acute ischemic stroke endovascular treatment by thrombectomy. Close cooperation of stroke neurologist with interventional radiologist allowed appropriate selection of patients with acute cerebral infarction who could receive primary treatment after neurological and radiological evaluation. All included patients followed strict indications, contraindications and time window was less than 8 hours for anterior circulation and less than 24 hours for basilar artery occlusion. (1;3) One of the inclusion criterion was artery occlusion that usually leads to large neurological impairment, severe inability or death - occlusion of carotid artery, middle cerebral artery M1 segment or basilar artery. Despite relatively small number of patients, successful endovascular mechanical thrombectomy was demonstrated in 92% that is similar to literature data. (8;10-12) In fact, only small number of patients had complications after thrombectomy like symptomatic postprocedural intracerebral hemorrhage, distal thrombembolic events during revascularization, unsuccesfull revascularization or death, which can be explained by many factors including the procedure by itself. The most common complication was
middle cerebral artery M1 segment occlusion (49%) but isolated carotid artery occlusion was observed only in 5%. Our study represents positive effect of endovascular thrombectomy in patients with acute cerebral infarction. Most of patients had positive outcome and minimal or no neurological inability at the discharge moment (NIHSS <8). Thereafter, this study confirms the efficacy and success of acute ischemic stroke endovascular treatment by thrombectomy.

CONCLUSIONS

These results suggest that endovascular revascularization for patients with acute ischemic stroke and large artery occlusion is relatively safe and effective with good outcome for those stroke patients who can't benefit from i/v thrombolysis.

Conflict of interest: None

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ORIGINAL ARTICLE

Possible Neurosurgical Contribution in Treatment of Ischemic Middle Cerebral Artery (MCA) Stroke

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Summary
Introduction. Malignant middle cerebral artery territory infarction is the most devastating form of ischemic stroke and its treatment is still a controversial issue. Recently there has been a resurgence of interest in hemicraniectomy as a treatment tool.
Aim of study. The aim of study was to estimate potential group of patients who could be considered as a candidates for decompressive surgery.
Materials and methods. Retrospective analysis of 748 patients hospitalized in Pauls Stradins Clinical University Hospital with diagnosis of MCA stroke was performed. Patients were divided into two groups of severe and mild stroke. Groups were subdivided depending whether there was or there was no radiological and/or clinical evidence of space-occupying edema. All subgroups were analyzed in terms of mortality, cause of death.
Results. Study demonstrated higher mortality rate in group of severe MCA stroke and radiological evidence of space occupying edema was much higher in this group as well. Most of deaths in the group of severe stroke were associated with brain herniation.
Conclusion. Estimated number of patients to be considered as candidates for decompressive surgery (those who die due to fatal brain edema) in our institution is 75 per year or about 10% of all MCA strokes admitted.
Key words: malignant ischaemic stroke, space-occupying edema, decompressive surgery

INTRODUCTION

Despite great improvements in the management of ischemic stroke, it is still one of the leading causes of death worldwide[1]. So-called "malignant" middle cerebral artery (MCA) territory infarction is the most devastating form of ischemic stroke that carries mortality rate up to 80% (2, 3, 5, 6, 11, 12, 13) and produces severe disability among survivors. Death or neurological devastation results from progressive edema of the massive (more than 50% of MCA territory) infarct, brain tissue shifts, compartmentalized elevation of intracranial pressure (ICP), extention of ischemia to adjoining vascular territories, and brain herniation. Life-threatening edema usually becomes manifest between the second and fifth day after stroke onset (5, 11) (but in some cases patients may deteriorate from mass effect on first day or later than in week, but also can manifest as early as within the first 24 hours (8)) and prognosis for these patients is poor despite maximal conventional medical therapy according to the best medical practice. Such massive hemispheric infarctions occur roughly in 10% (2, 5, 12, 13) of all ischemic strokes.

The treatment of this kind of infarct is still a controversial issue in neurology and neurosurgery. Medical therapies (including endotracheal intubation, osmotherapy, hyperventilation, blood pressure control, barbiturates and steroids) aimed at reducing edema have not proven to reduce mortality or disability (2, 5, 6, 11, 12). Several reports suggest that these therapies may be ineffective or even detrimental (5).

Because of the limitations of medical therapies, decompressive hemicraniectomy with duraplasty has been proposed as surgical option for those experiencing large volume, space occupying MCA stroke. The rationale of this therapy is to create compensatory space to accommodate the swollen brain, thus preventing the death spiral (vicious circle of extensive edema and further infarction) by normalizing intracranial pressure, restoring compromised flow in penumbra and adjacent vascular territories, and restoring the midline position of the brain stem and diencephalon.

The procedure is not new (4, 9), but was performed rarely for MCA stroke before the 1990s, primarily because of concerns that it would result in survival with overwhelming neurological impairment and handicap, however, with improvements in postoperative critical care, there has been a resurgence of interest in hemicraniectomy over the past 15 years. Results of recently done randomized controlled trials (RCTs) (DECIMAL, DESTINY and HAMLET) exploring the impact of early decompressive surgery on mortality and functional outcome after malignant stroke in selected population clearly show increased probability of survival without increasing the number of very severely disabled (modified Rankin Scale (mRS) of 5) survivors (5, 6, 11, 12). On the basis of data from the first randomised controlled trials, neurosurgical team of Pauls Stradins Clinical University Hospital has started to perform decompressive hemicraniotomies in selected patients of

"malignant" MCA stroke with similar inclusion criteria to RCTs which are mentioned above.
However, surgical treatment of "malignant" MCA stroke as a last resort is challenging task that requires combined multi-team approach with involvement of ICU, neurosurgical and many other services. In order to identify cost effective treatment options and to plan resources that are needed to undertake flow of such a difficult group of patients, there is a great need of estimation of approximate amount of patients per year that would potentially require such a treatment.

AIM OF THE STUDY

The aim of study was to estimate the potential group of patients who could be considered as a candidates for decompressive surgery.

MATERIALS AND METHODS

Patients for the study were selected on the basis of final clinical diagnosis of MCA stroke. Retrospective analysis of clinical notes from January to December 2010 was performed in Pauls Stradins Clinical University Hospital. Patients of any age and sex were included. The data collected by authors included age and sex of patient, severity of symptoms of stroke, whether there was or there was no evidence of space-occupying edema (SOE) in CT or MRI scans at any time of period of treatment. Depending from severity of symptoms all patients were divided into two groups – 1) severe MCA stroke and 2) mild MCA stroke. We defined MCA stroke as severe if patient had score of at least 7 in NIH Stoke Scale motor evaluation (plegic in one extremity and with deep weakness in another on the same side) at presentation. If the motor deficit was milder patient was defined as mild MCA stroke. Both groups were subdivided by criteria whether there was or there was no radiological evidence of SOE in CT or MRI. As evidence of SOE we defined compression of lateral ventricles with or without midline shift, with or without compression of basal cisterns. Overall mortality rate, mortality rates by groups and subgroups were taken into account. Based on clinical notes cause of death was investigated among those who died. Windows Excel software was used for data processing and analysis.

RESULTS

There were 748 patients included the study. The mean age of the patients was 73.9 years (range from 37 to 97 years); 464 (62%) were women. The overall mortality rate was 12.56%.
There were 548 (73.26%) patients in group of mild stroke with 2.6% (n=14) mortality rate and 200 (26.74%) patients in group of severe stroke with 40.0% (n=80) mortality rate.
In the group of mild MCA stroke there was found radiological evidence of SOE in 22 cases (4.01%) and none of them died. In other 526 cases there was no evidence of SOE. Most of the deaths (n=9) in this subgroup were related to comorbidities (pulmonary embolism, myocardial infarction etc.) and in other 5 cases the cause of death was not clearly identified.
In the group of severe MCA stroke there was found evidence of SOE in 97 cases with 51.6% (n=50) mortality rate. Analysis of clinical notes suggests that 49 of those 50 patients died due to brain herniation. There was no evidence of SOE in other 103 cases with 29.1% (n=30) mortality rate. Nonetheless analysis of clinical notes suggests that death in most of the cases (n=26) in this group was related to brain herniation and the rest were due to comorbidities.

DISCUSSION

Data from internationally available literature indicates that mortality rate after "malignant" MCA stroke can be up to 80%. It is not precisely specified in literature whether all of "malignant" MCA stroke patients die from brain herniation or there is still a subset of patients which die from complications which are not directly related to herniation, but have developed on the background of extremely devastating disease. It is known that the distribution of mortality rates is bimodal, with an early peak within the first 3-6 days (due to transtentorial herniation of the brain), followed by a second peak during the 2nd and 3rd weeks after stroke (a result of complications related to both hospitalization, such as pneumonia, as well as medical comorbidities, such as myocardial infarction and hearth failure) (10). So this might also partially explain why decompressive hemicraniectomy performed in case of "malignant" MCA stroke as a life saving procedure still carries roughly 20% mortality (12). In this study we did not analyze mortality rate of proven "malignant" MCA stroke, but tried to estimate group of patients which are at high risk of developing fatal brain edema. In our study we found only 40% mortality rate in severe stroke group and this can be easily explained by fact that not every patient with an MCA infarct develops fatal brain edema, and the factors that contribute to a malignant course are poorly understood (6).
Despite the fact that severity of symptoms not always is equivalent to volume of infarction, still it is well known that one of the most consistently identified clinical risk factors for death (due to brain herniation) after MCA infarction is a high initial NIH Stroke Scale Score (if which motor evaluation along with coexisting stroke symptoms makes a big part). All randomised controlled trials focusing on surgical treatment of "malignant" MCA stroke as almost only one of the clinical inclusion criteria has a clinical deficit suggestive infarction in the territory of the MCA with high initial NIHSS score (6, 12). This can explain a good correlation between severity of symptoms and mortality in our analysis.
Hemicraniectomy is aimed to save lives of patients which have developed fatal brain edema. According to the protocol of Pauls Stradins Clinical University Hospital, CT scan is typically done at the first presentation of patient in hospital after sudden onset of neurological deficit, just to rule out diagnosis of intracerebral haemorrhage. In most of the cases CT scan is not repeated. It is well known that SOE usually manifests itself between

second and fifth day after stroke onset (6, 11). Analysis of clinical notes suggests that most of those 30 patients who died in the group of severe stroke with no evidence of SOE still died due to brain herniation. The lack of radiological evidence of SOE can be explained by fact that scans were not repeated in few days after onset of disease when edema could be seen.

When summarizing patients in the severe stroke group with evidence of SOE (both clinical and radiological), it makes all together 75 patients or 10.02% of all MCA stroke patients.

The analysis of RCTs confirms suggestions that decompressive surgery undertaken within 48h of stroke onset reduces mortality and increases the number of patients with favourable functional outcome after malignant hemispheric infarction at least if they are age of 60 or younger and had no significant coexisting diseases (12). At the moment there is no evidence from RCTs exploring impact of decompressive hemicraniectomy on patients over age of 60. Although age is powerful predictor of bad outcome (several uncontrolled series demonstrated improved outcomes in patients under age of 50 or 60 (1, 2, 7), decompressive surgery can be useful also for selected patients older than 60 years of age (patients without significant comorbidities that could limit their survival and/or rehabilitation potential).

CONCLUSIONS

From our study it seems that severity of motor symptoms of MCA stroke can be a valuable prognostic tool helping to identify those MCA stroke patients who can benefit from decompressive surgery. Estimated number of patients to be considered as candidates for decompressive surgery (those who die due to fatal brain edema) in our institution is 75 per year or about 10% of all MCA strokes admitted.

Conflict of interest: None

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ORIGINAL ARTICLE

Uterine Prolapse: Immunohistochemical Study of the Pelvic Ligaments

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Summary
Introduction. Uterine prolapse is a pathological condition when pelvic support system loses elasticity and the uterus descends down into the vagina, leading to pain and even protrusion of tissue from the vagina.
Aim of the study. Compare morphology of pelvic ligaments in women with uterine prolapse and without it.
Materials and methods. Biopsies of *lig. teres uteri* were taken during the reconstructive surgery from 7 women with uterine prolapse and the control group of 3 women. Tissues were stained with hematoxilin and eosin, periodic acid – Schiff method and with antibodies of bFGF, FGFR 1, VEGF, PGP 9.5, Collagen III and IV, MMP-9, microscoped at 400X magnification and evaluated semiquantitatively. Data were analysed using non-parametric statistics – Mann – Whitney U test.
Results. VEGF was statistically significantly increased (U = 3.5, p = 0.022, two-tailed Mann – Whitney U test) in the prolapse group, in comparison to the control group. Other parameters did not display any statistically significant difference when comparing the two groups, however, amount of GAGs stained with periodic acid – Schiff method showed a notable tendency to decrease in the prolapse group in comparison to the control group.
Conclusions. Increased number of VEGF positive endothelium indicates hypoxia and stimulation of angiogenesis in female pelvic ligaments with uterine prolapse. Tendency of GAGs to decrease in the pelvic ligaments of females with uterine prolapse suggests qualitative degradation of tissue.
Key words: uterine prolapse, immunohistochemistry, vasoendothelial growth factor; glycosamynoglycans.

INTRODUCTION

Pelvic organ prolapse is a descent of the uterus down into the vagina or a descent of vagina itself. The condition is graded depending on the level of the descent of the uterus: to the upper vagina (1st degree), at the vaginal opening (*s. introitus*) (2nd degree) and *procedentia* (the uterus and the cervix are both prolapsed out of the *introitus*) (3rd degree). Vaginal prolapse may be 2nd or 3rd degree. Symptoms of the 1st degree prolapse are minimal, however 2nd and 3rd degree prolapses present with pain, pressure and a sensation of "organs falling out". Vaginal mucous may be chronically inflamed, secondarily infected and ulcerated. Urinary incontinence may also follow. Severe symptoms require surgery, for example, hysterectomy with a repair of pelvic support structures. One study shows that prevalence of symptomatic uterine prolapse is 3.6% within women aged 51-60 years, and that up to 15% of hysterectomies are administered due to this condition (5).

Up to this day pathogenesis of the uterine prolapse is not fully understood. Risk group consists of women in menopausal period of life and women who have experienced delivery more than once (2, 5, 7). Several studies (6, 7, 9, 10) state that this could be explained by the deformation of the pelvic support system, especially, *lig. latum uteri*, *lig. teres uteri*, because of the mechanical stress that these ligaments are exposed to during delivery and pregnancy. Hormone deficiency during menopause is also considered a major etiological factor (7).

Ligaments are a type of dense regular connective tissue, which mostly consists of the extracellular matrix (ECM). The ECM incorporates, primarily, collagen, elastic fibers and glycoproteins. Collagen type I-III are inextensible fibers responsible for the tensile strength of the tissue. Collagen type IV, however, forms a non-fibrous structure as support for a more flexible basal lamina (7). These structures as well as matrix metalloproteinases (MMP) are mainly produced by fibroblasts. MMPs are responsible for proteolytic degradation of collagens, which is a crucial factor for tissue remodeling, for example during the healing process after tissue damage induced by delivery. A clot forms around the damaged area, cells within the clot release growth factors and other molecules. This leads to an invasion by neutrophils and macrophages, which begin phagocytosis of necrotic tissue. This process is followed with yet another release of growth factors that stimulate fibroblast proliferation. Granulation tissue is formed by fibroblasts. This tissue consists of irregular collagen, glycosaminoglycans, it is highly vascularised. Slowly ECM becomes more organized. There is a decrease in cellular content and an increase in collagen type I, which cross-links it to healthy tissue. Repaired tissue is always mechanically weaker than undamaged ligaments (11).

Majority of researches focus on studying three directions in order to explain the pathogenesis of the uterine prolapse: changes in morphology of the connective tissue in the uterine supportive system (6, 7, 9, 11), the

roles of growth factors in this process (3, 8, 10), changes in innervations and vascularization of the ligaments after the prolapse (7, 8, 10).

Several studies focus on comparing glycoproteins and collagen in healthy women and women with prolapse. Scientific reports are conflicting on this matter, but the majority tends to note the decreased ratio of I/III collagen in women with the uterine prolapse, thus the reduction of type I collagen is compensated by the increase in type III collagen (10). However, another study states that the genital prolapse has an association with a moderate reduction of collagen type III (7). Women with a uterine prolapse have an increased expression of MMP-9 (11). Increase in collagen type III together with the expression of MMP-9 is a feature of tissue that is remodeling (7). Concentration of glycosaminoglycans (GAGs), a mucopolysaccharide that is bound to a core protein to form proteoglycans, seems to be decreased in the parametrium of postmenopausal women (the uterine prolapse risk group) (13). However, no significant change in the amount of GAGs in pelvic ligaments of women with uterine prolapse was observed, when comparing to the control group (1).

A review article (10) and several other studies (3, 8, 13) were dedicated to the roles of growth factors in ligament healing. A significant factor in stimulation of angiogenesis and cell migration and proliferation is the Basic Fibroblast Growth Factor (bFGF). *In vivo* studies have shown that application of bFGF to an injured ligament correlates with the increase of collagen type III expression and cellular proliferation. Application of bFGF reduces expression of type I procollagen, and that bFGF has a significant impact only on the formation of granulation tissue, afterwards it effects the healing tissue less potently (13). The bFGF mediates its effects through an interaction with cell surface receptors, for example, fibroblast growth factor receptor 1 (FGFR1). Unfortunately, there were no scientific studies found, concerning the expression of these receptors in pelvic ligaments.

Vascular Endothelial Growth Factor (VEGF) is most active during the proliferative and remodeling phases. It is actively expressed during such stimuli as hypoxia and other growth factors, leading to angiogenesis when blood vessels are produced by sprouting from existing vasculature. Cells around the injured tissue express VEGF, which has an effect on endothelial cells, by stimulating proliferation, differentiation and migration (8). In response to VEGF endothelial cells express MMP-9, which degrades the basal lamina, allowing endothelial cells to migrate and align further into injured tissue, forming anastomosis with other vessels (11). VEGF also increases microvascular permeability, thus stimulating inflammation (10).

Protein gene product (PGP 9.5) is a hydrolase izoenzyme and is frequently used as a neural marker. Its antibody usually stains peripheral nerves and ganglia. This technique was used in one study to evaluate the nerve density in the uterine ligaments in women with prolapse. The nerve density was diminished in women with the uterine prolapse in relation to the control group (10).

AIM OF THE STUDY

Purpose of this study was to focus on the immunohistochemical detection of growth factors, their receptors, matrix degrading enzymes, neuropeptide-containing innervation and tissue ischemia and quality proteins in the pelvic ligaments of females with uterine prolapse, and their comparison to pelvic ligaments of females without uterine prolapse. This will allow us to understand the pathophysiological processes involved in uterine prolapse more clearly.

MATERIALS AND METHODS

Biopsies of *lig. teres uteri* were gained during reconstructive surgeries of 6 women with 3rd degree uterine prolapse and 1 woman with a 2nd degree vaginal prolapse, thus the prolapse group consists of 7 women (N_1). Control group consisted of 3 women (N_2). Biopsies of *lig. teres uteri* were taken during surgical procedures unrelated to pelvic organ prolapse, including 2 cases of uterine leiomyoma and 1 case of an ovarian cyst (Table 1).

Ligaments were stained with hematoxilin and eosin and immunhistochemically with antibodies of basic Fibroblast growth factor (bFGF, 1:200 solution, Abcam, England), Fibroblast growth factor's receptor 1 (FGFR1, 1:100 solution, Abcam, England), Vasoendothelial growth factor (VEGF, 1:50 solution, Santa Cruz, USA), Protein gene product 9.5 (PGP 9.5, 1:600 solution, DakoCytomation, Denmark), Collagen III (Col III, 1:50 solution, Quartett, Germany), Collagen IV (Col IV, 1:30 solution, Invitrogen, USA) and with matrix metalloproteinase 9 (MMP-9, 1:100 solution, R&D systems, Germany). Materials were also stained by periodic acid – Schiff method (PAS kit 04-130802, Bio-optica, Italy) in order to localize glycosaminoglycans and glycoproteins.

Specimens were microscoped and photographed at 200X, 250X, 400X magnification, and evalued semiquantitatively. Depending on the realtive quantity of positively stained structures, specimens were rated as having a few positive structures (+), moderate quantity of positive structures (++), numerous quantity of positive structures (+++) and abundant amount of positive structures (++++).

Data analysis was conducted using Statistical Package for the Social Sciences (SPSS) program version 18.0. Results of semi-quantitative evaluation were transformed into numerical form, where + is equal to 1, ++ is equal to 2, +++ is equal to 3, ++++ is equal to 4 and a negative result is equal to 0. The prolapse group was compared to the control group using non-parametric statistics, specifically Mann-Whitney U test. Two-tailed P values of ≤ 0.05 were considered as statistically significant.

RESULTS

Specimens in the prolapse group had signs of acute and chronic inflammation. No significant abnormalities of

tissue were acknowledged in the control group. More specifically prolapse group patients 1, 2 and 3 had sclerotic blood vessels. Patient 4, 6 and 7 had marked proliferation of fibrous connective tissue. Patient 5 and 7 had signs of edema, dilated veins and an infiltration of macrophages. Control patient 1 had dense fibrous connective tissue with no signs of infiltration.

VEGF was expressed by the endothelium in both groups (Table 1, Figure 1 and 2). VEGF was statistically significantly increased ($U = 3.5$, $p = 0.022$, two-tailed Mann – Whitney U test) in the prolapse group, where there was a moderate quantity of positive structures (Table 2), in comparison to the control group, where results ranged from weakly to moderately positive.

Number of bFGF, FGFR1, PGP 9.5, Collagen III and IV, MMP – 9 antibodies, and structures strained with periodic acid – Schiff method did not confirm any statistically significant difference between the prolapse group and the control group (Table 2). However, there was a notable tendency.

Quantity of FGFR1 positive lemmocytes and fibroblasts was increased in the prolapse group (moderate to numerous quantity of positive structures), in comparison to the control group (moderate quantity of positive structures), ($U = 6.0$, $p = 0.199$, two-tailed Mann – Whitney U test).

Quantity of collagen IV positive structures in the perineurium and the basement membrane of the endothelium was increased in the prolapse group (moderate quantity of positive structures), in comparison to the control group (a few to moderate quantity of positive structures), ($U = 4.0$, $p = 0.129$, two-tailed Mann – Whitney U test).

Periodic acid – Schiff method allowed to identify glycosaminoglycans (GAGs) in the connective tissue of the ligaments (Figure 3 and 4). Amount of GAGs was decreased in the prolapse group (numerous quantity of positive structures), in comparison to the control group (numerous to abundant quantity of positive structures), ($U = 4.5$, $p = 0.156$, two-tailed Mann – Whitney U test).

Number of PGP 9.5, collagen III, bFGF, MMP-9 positive structures had neither a notable tendency, nor statistically significant difference when comparing the prolapse group to the control group.

DISCUSSION

The study has demonstrated a statistically significant increase of VEGF, which was identified in the endothelium in the pelvic support ligaments (*lig. teres uteri*), in patients with uterine prolapse. There were no other studies found that had researched the relative quantity of VEGF in the pelvic support ligaments during pelvic organ prolapse, but several studies show that VEGF is a marker of hypoxia and angiogenesis in connective tissue (11). In other words amount of VEGF is usually increased in regions where blood supply was stopped or decreased, for example due to trauma or mechanical damage, in order to restore blood flow by producing blood vessels' collaterals.

VEGF usually mediates expression of MMP-9, which degrades the basal lamina of blood vessels, thus allowing migration of the endothelial cells (3), followed by proliferation of type III collagen (10). Neither increased number of MMP-9 positive structures, nor type III collagen positive structures in comparison to the control groups was acknowledged in this study. It is crucial to highlight that some studies have stated that type III collagen can be also decreased during pelvic organ prolapse (4). Therefore no correlation between uterine prolapse and the amount of type III collagen was identified.

Even though no statistically significant decrease in the number of glycosaminoglycans can be seen in the prolapse group, the results (Table 1) clearly show that 3 out of 7 patients in the prolapse group had a few to moderate amount of positive periodic acid – Schiff reactive structures, whereas all patients in the control group had a numerous to abundant amount of positive structures. This allows us to conclude that there is a notable tendency for GAG to decrease in patients with uterine prolapse, and that by increasing the number of patients that participate in this study in both - the control group and the prolapse group – the difference may be proved statistically significant. This is a sign that mechanical durability of ligaments in patients with uterine prolapse is also decreased.

CONCLUSIONS

1. Number of VEGF positive endothelial cells is increased in *lig. teres uteri* in patients with uterine prolapse, suggesting notable presence of tissue hypoxia and stimulation of angiogenesis.
2. The amount of GAGs has a notable tendency to decrease in *lig. teres uteri* in patients with uterine prolapse, suggesting qualitative degradation of tissue.

Table 1. Summary of the semi-quantitative results of the immunohistochemical and periodic acid – Schiff methods per each patient separetly and comparison of both groups

Immunhistochemical study:

Uterine prolapse group									Control group			
	1	2	3	4	5	6	7	Σ	1	2	3	Σ
Age	66	69	72	73	60	76	68		45	70	43	
Pathology	pelvic organ prolapse								Leimyoma	Ovarian cyst	Leiomyoma	
FGFR1	+++	++	+++	++	++	++	+++	**++/+++**	++	++	++	**++**
bFGF	0/+	0/+	+	+	0/+	0/+	+	**0/+**	+	0/+	0/+	**0/+**
VEFG	++	++	++	++	++	++	++	**++**	++	+	+	**+/++**
PGP 9.5	+	++	+	++/ +++	0/+	++	++	**+/++**	++	++	++	**++**
collagen III	+++	++++	+++	+++	+++	+++	++++	**+++/ ++++**	+++	++++	+++/ ++++	**+++/ ++++**
collagen IV	++	++	+++	+/++	0/+	++/ +++	+/++	**++**	+	+/++	+/++	**+/++**
MMP 9	0/+	+	0/+	++	++	++	0/+	**+/++**	++	+	+	**+/++**
Periodic acid - Schiff method:												
GAG	+++	++++	++	++++	+++/ ++++	+/++	++	**+++**	++++	+++/ ++++	++++	**+++/ ++++**

(+ - a few positive structures, ++ - moderate quantity of positive structures, +++ - numerous quantity of positive structures, ++++ - abundant quantity of positive structures).

Table 2. Data analysis results using non-parametric statistics (Mann – Whitney U test). VEGF displays statistically significant difference (p<0.05).

Mann - Whitney U test (two-tailed):

	Mean Ranks		**P value**	**U score**
	Prolapse group	Control group	≤0.05	
Patients	N_1=7	N_2=3		
VEGF	6.50	3.17	**0.022**	3.5
FGFR1	6.14	4.0	0.199	6.0
bFGF	5.64	5.17	0.789	9.5
PGP 9.5	5.07	6.50	0.439	7.5
collagen III	5.07	6.50	0.434	7.5
collagen IV	6.43	3.33	0.129	4.0
MMP 9	5.21	6.17	0.629	8.5
GAG	4.64	7.50	0.156	4.5

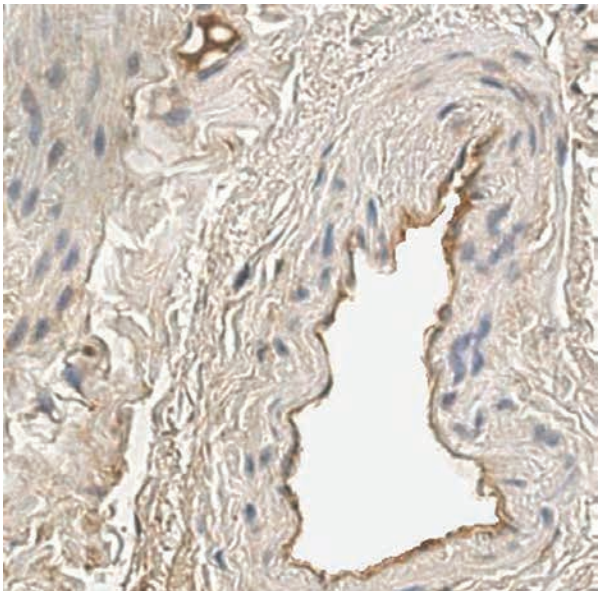

Fig. 1. VEGF in the endothelium, patient 1 with uterine prolapse, x400

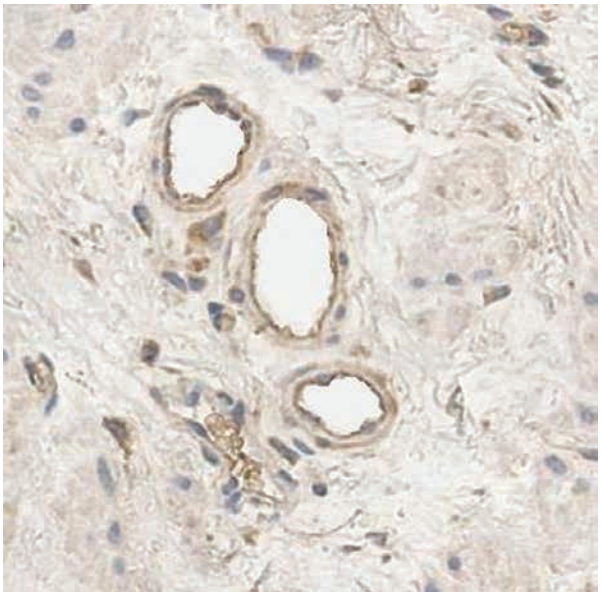

Fig. 2. VEGF in the endothelium of venules, control patient 3, x400

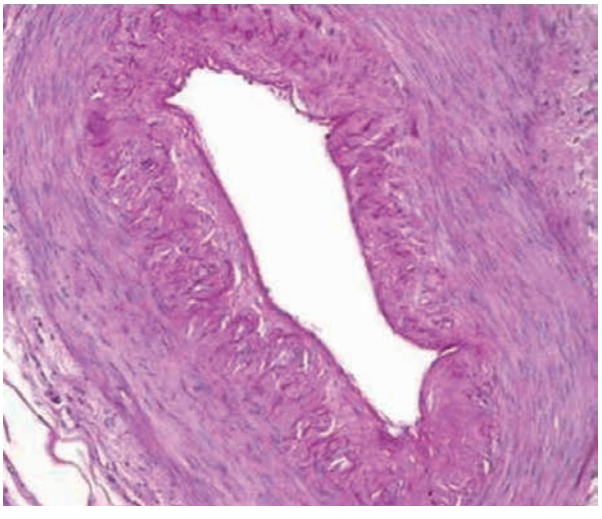

Fig. 3. Periodic acid – Schiff reaction. GAGs in the artety of patient 4 with uterine prolapse, x200.

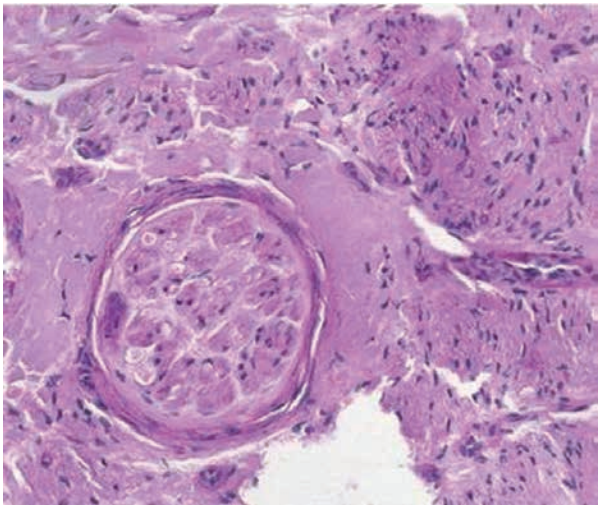

Fig. 4. Periodic acid – Schiff reaction. GAGs in the perineurium of patient 5 with uterine prolapse, x400

Conflict of interest: None

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ORIGINAL ARTICLE

Il-6 and Il-10 Expression in Brain Tissue in Children and Adults after Fatal Traumatic Brain Injury

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Summary
Introduction. One of the leading secondary damage processes determining the outcome of head injury is cerebral ischemia. The inflammatory reactions are important factors in cases of ischemic brain damage. Some studies characterize generalized reactions of cytokines in brain, but there are less studies about inflammatory reactions in the determined areas of the brain in different time points.
Aim of the study. To determine the reaction of brain tissue after fatal brain injury in different time points after the trauma, by detecting IL-6 in the pyramidal neurons of CNS gray matter (GM) and in the white substance (WS) and IL-10 in the WS of the impact and counterstroke areas.
Materials and methods. We used brain tissue material from the trauma and counterstroke spots of 11 patients died after fatal traumatic brain injury in different time points. Brain tissue specimens were routinely fixed, embedded into paraffin, cut in 5 μm thick slides. For immunohistochemistry we used monoclonal antibodies against recombinant IL-6 of human origin and polyclonal rabbit antibodies against IL-10 of human.
Results. There were found statistically significant differences in IL-6 positive neurons in the GM, and IL-6 and IL-10 positive glial cell numbers in the WS in the spot of counterstroke and in the spot of direct impact in both patient groups (Mann-Whitney U Test $p \leq 0{,}001$ for all groups).
Conclusions. The IL-6 and IL-10 positive glial cell numbers correlate with the outcome of trauma. The inflammatory reaction in the WS in the spot of counterstroke was more marked than in the spot of direct impact. The activity of inflammatory reaction depends on the time period after the traumatic event.
Key words: Traumatic brain injury, cerebral ischemia, secondary brain damage, cytokine IL-6, cytokine IL-10, inflammatory reactions.

INTRODUCTION

For several decades head injury (HI) is one of the leading causes in the world of death and disability. The main pathological aspect determining the outcome of HI is cerebral ischemia (CI), one of the leading secondary damage processes that develop straight after the primary damage. It is a great challenge to understand the process, causing ischemic brain damage, and to investigate the possibilities for delaying the process of cerebral ischemia. It has been proven that inflammatory reactions are important factors in cases of ischemic brain damage, after the primary damage in case of trauma. Reviewing the pathological processes from this point of view allows understanding the inflammatory process in the brain, and potentially affecting the treatment of cerebral ischemia (5).

Central nervous system (CNS) is able to realize a complex inflammatory cascade as a response to an injury through cytokines, macrophages and glial cell (predominantly astorcytes and microglia). Since it has been revealed that cytokines activate glial cells *in vivo* (1), and glial cells produce cytokines as a response to external impulses *in vitro* (8;16;21), close connection between inflammation, production of cytokines and gliozes has been established.

It is proven that several cytokines, e.g., IL-1β, IL-4, IL-12, TNFα are taking part in neurotoxic reactions and promote damage of the cortical neurons (11;4;23). Whereas groups of other cytokines ensure neuro-protection, this group includes IL-6 and IL-10. The studies of previous years characterize generalized reactions of cytokines in brain or cerebrospinal fluid, but there are less studies about inflammatory reactions in the peculiar spot of the injury in the brain, for example, in the spot of the impact and counterstroke in persons died in the spot of the accident, and in those who died several hours or days after the injury.

AIM OF THE STUDY

Aim of our study was to determine the reaction of brain tissue after fatal brain injury in different time points after the trauma, by detecting IL-6 in the pyramidal neurons of CNS gray matter and in the white substance and IL-10 in the white matter of the impact and counterstroke areas.

MATERIALS AND METHODS

We used material from the archive of Institute of Anatomy and Anthropology in Riga Stradins University (RSU) brain tissue from the trauma and counterstroke

spots of 28 patients (permission of the RSU Committee of Ethics Nr. E-9(2) - 17.12.2009.). All patients had severe head injury (SHI). Patients with multiple traumas were excluded. In the beginning all 11 patients were divided in 2 groups: 3 patients, which died in the place of accident (all of them adults), and 8 patients, which survived and received therapy for different amounts of time 5 children hospitalized and received therapy for more than 24 hours (a 2-year-old, hospitalized for 48 hours, a 16-year-old, hospitalized for 78 hours, another 16-year-old, hospitalized for 28 hours, a 17-year-old, hospitalized for 36 hours and another 17-year-old, hospitalized for 168 hours) and 3 adults hospitalized after SHI and received therapy for more than 24 hours (a 36 year old, hospitalized for 15 days (360 hours), a 37-year-old, hospitalized for 11 days (264 hours), and a 40-year-old, hospitalized for 7 days (168 hours).

In both groups we analyzed cell count with positive interleukin IL-6 in pyramidal neurons of CNS gray matter, glial cell count of CNS white matter with positive interleukin IL-6 and glial cell count of CNS white matter with positive interleukin IL-10 in the areas of impact and counterstroke. Brain tissue specimens were fixed in 10% formaldehyde. Then tissue specimens were embedded into paraffin, cut in 5 μm thick slides. For immunohistochemistry we used monoclonal antibodies against recombinant IL-6 of human origin (IL-6 (NYRhIL6): sc-73319 Mouse Monoclonal antibody, working dilution 1:100, Santa Cruz Biotechnology, USA) and polyclonal rabbit antibodies against IL-10 of human origin (Rabbit polyclonal to IL-10, ab34843, working dilution 1:400, Abcam, UK).

Sections were de-parafinised in xylene, and kept in absolute ethanol, then rinsed with PBS pH 7,4 (10 min), put into 4% citrate buffer solution, and placed in microwave for 20 min (750W). After cooling and rinsing with PBS tissue samples were covered in 150 μl 3% hydrogen peroxide (10 min.). After rinsing with PBS the primary antibody (30 μl) was applied for 2 hours, then LSAB + LINK (linked streptavidin antibody) was applied for 30 min, LSAB + KIT (streptavidin connected with enzyme peroxidase) was applied for 25 min, and DAB color reaction for 10 min. Finally, routine hematoxyline and eosine staining was performed in each sample (05B1003 Eosin Y Alcoholic Solution and 05M06002 Mayers Hematoxylin, Bio optica, Italy).

Further data processing was done with SPSS (Statistical package for social sciences for Windows 17.0 ASV) software, using non-parametrical statistical method, group and correlation methods.

RESULTS

In the overview slides tissue in the area of impact were relatively less damaged than in the area of counterstroke. Plethoric blood vessels and glial proliferation was noted in the surviving patients. In all groups glial edema was seen.

In 3 patients, died in the spot of accident the count of IL-6 positive pyramidal neurons in the cerebral cortex in the spot of the impact was less than in the spot of counterstroke - the median cell count (MCC) was 33, interquartile range (IQR) was 14,5 (18<42), and MCC was 44, IQR -10,5 (34<53), respectively. (see Figure 1). Also the 8 survivors the number of IL-6 positive pyramidal neurons in the cortex in the spot of impact was less than in the spot of counterstroke MCC was 34,5 IQR – 17,8 (6<49) in the spot of impact, and in the spot of counterstroke it was 41, IQR – 27,8 (13<67). (see Figure 1). Comparing both groups reveals higher number of IL-6 positive pyramidal neurons in the spot of counterstroke in those who died immediately after accident, but there was no statistically significant difference. (Mann-Whitney U Test, $p = 0.890$). There were also no statistically significant differences in IL-6 positive pyramidal neurons in the spot of impact between these two groups. (Mann-Whitney U Test $p = 0.827$). Comparing two dependent selections statistically significant differences between the IL-6 positive neurons in the spots of counterstroke and direct impact were found between survivors (Wilcoxon Signed Ranks Test, $p < 0,001$) and those died in the place of accident (Wilcoxon Signed Ranks Test, $p = 0,011$) (see Table 1).

Three patients of those who died in the place of accident had fewer glial cells in the white substance of the brain in the spot of impact (Figure 4) than in the spot of counterstroke (Figure 5), MCC was 76, IQR – 15 (67<91) in the spot of the impact, and MCC was 101, IQR – 25 (76<113) in the spot of counterstroke (Figure 2).

In 8 survivors has fewer IL-6 positive glial cells in the white substance of the brain in the spot of impact (Figure 6) than in the spot of counterstroke (Figure 7); MCC in the spot of the impact was 36, IQR – 9,3 (8<52), and MCC in the spot of counterstroke was 61, IQR – 23,5 (21<75) (Figure 2). The number of IL-6 positive glial cells in the group of the deceased patient was higher in both, spots of impact and counterstroke (see Figure 2). There was a statistically significant difference in IL-6 positive glial cell numbers in the white substance of the brain in the spot of counterstroke between the both patient groups (Mann-Whitney U Test $p < 0,001$); there was also a statistically significant difference in the white substance of the brain in the spot of direct impact in both patient groups (Mann-Whitney U Test $p < 0,001$). Comparing two dependent selections statistically significant differences between the IL-6 positive glial cells in the spots of counterstroke and direct impact were found between survivors (Wilcoxon Signed Ranks Test, $p < 0,001$) and those died in the place of accident (Wilcoxon Signed Ranks Test, $p = 0,038$) (see Table 1).

Three deceased patients had a smaller number of IL-10 positive glial cells in the white substance of the brain in the spot of direct impact (Figure 8) than in the spot of counterstroke (Figure 9), the MCC in the spot of the impact was 25, IQR – 13 (12<31), the MCC was 37, IQR – 18,5 (22<51), respectively (Figure 3). In the 8 survivors the number of IL-10 positive glial cells in the spot of direct impact (Figure 10) was smaller than in the spot of counterstroke (Figure 11), the MCC was 4 in the spot of impact, IQR – 5 (0<18), the MCC in the spot of counterstroke was 12, IQR – 9 (4<33) (Figure

3). Comparison of these two groups revealed that the number of the IL-10 positive glial cells in the deceased group was higher in both, spots of direct impact and counterstroke (see Figure 3). The difference between deceased patients and the survivor group in the number of IL-10 positive glial cells in the spot of counterstroke (Mann-Whitney U Test $p < 0,001$) and in the spot of direct impact (Mann-Whitney U Test $p < 0,001$) was statistically significant.

Comparison of two dependent revealed selections statistically significant differences between the IL-10 positive glial cells in the spots of counterstroke and direct impact were found between survivors (Wilcoxon Signed Ranks Test, $p < 0,001$) and those died in the place of accident (Wilcoxon Signed Ranks Test, $p = 0,050$) (see Table 1).

DISCUSSION

It is known that the range and severity if HI is determined by the primary damage, which is the direct damage by mechanical forces in cranial bones and in brain tissue, thus creating both focal and diffuse tissue damage. The secondary damage develops as a result of biochemical reactions after the primary damage. Its amount is determined by early hypoxia and/or hypotension during resuscitation, which results in CI (9;14;17). The secondary damage induces neuro-inflammatory processes that manifests as the characteristic immune response of the body with a special role for pro-inflammatory cytokines (6;7;19), chemokines and anaphylotoxins of complement. The biochemical processes (inflammatory, non-inflammatory (apoptosis) taking place after severe head injury are not fully understood. It is due to wide range of endogenous neurotransmitters and their participation in different biochemical reations in different time periods after the accident, ensuring both agonistic and antagonistic processes (15).

Historically central nervous system (CNS) was considered to be immunologically privileged because of its special segregation from peripheral circulations by blood-brain barrier (BBB). In the last years it has been proven that CNS itself is a rich source of inflammatory mediators. The cells residing in brain – neurons, astrocytes, miroglial cells and macrophages can produce any mediator that is found in peripheral immune system, cytokines included (12). It is assumed that CNS has physiological immune supervision and that in cases of brain injury strong immune response reaction is initiated. Cytokines are the main or central mediatiors of the neuro-inflammatory reaction (6). The past years have shown a controversial conception about the dual nature of neuro-inflammatory reaction, which is marked as neurotoxin and neuro-protective action of inflammatory mediators, which is dependent on the expression of the mediators in time unit after the trauma (18;20). Our study focused on two cytokines: IL-6, which has the dual action, and IL-10, which has been proven to have neuro-protective characteristics (2). Mostly these studies have been performed in animals. Our study investigates material of 2 patient groups – deceased in the spot of accident, and survivors. It is remarkable that there were no significant difference in IL-6 positive cortical pyramidal neuron count between deceased and survived patients. But the reaction in the white substance, where glial cells, especially microglia that has an important role in the phagocytosis of the damaged brain tissue, take active part in inflammatory reaction, is more marked. (24). Comparison of IL-6 positive glial cells in both groups reveals more cells in both, direct impact and counterstroke spots in the deceased group. The same could be observed with IL-10 positive glial cell count in the white substance of the brain. The number was higher in the deceased patient group in both, direct impact and counterstroke spots. These observations could be explained by active astrocyte involvement in the inflammatory response - forming of a glial scar. The glial scar rapidly is formed after a trauma, when the spot of secondary damage starts rapid production of cytokines straight after primary damage (1). As the number of IL-6 and IL-10 positive cells was higher in survivors, it could be assumed that the most active immune response of healthy glial cells and penumbre glial cells happens straight after the primary damage as an inflammatory reaction. The glial scar is formed around the primary damage for protection of intact CNS tissue from the secondary damage (3). Also the experimental neuro-trauma models have shown reactions of angiogenesis and gliosis, induced by IL-6 (25). If our study showed the site of counterstroke to be the most active pro-inflammatory zone with remarkably more IL-6 positive glial cells, then a study performed on rats revealed more active pro-inflammatory zones to be in the spot of direct impact and the penumbre (10).

Our study investigated only two cytokine (IL-6 and IL-10) reaction in the gray and white matter of the brain in cases of fatal head injuries, when patients died in different time periods after the trauma. The patho-physiological processes in the brain tissue after HI are a very complex process, which is still unclear. Large-scale studies are being conducted for more accurate definition of the role of cytokines in the neuro-inflammatory processes. It is necessary to define the nature of every mediator, depending on time after the traumatic event, and interactions between the mediators themselves, also their impact on brain tissue, especially to the penumbre zone in different time units after the traumatic event (22;13). The better our understanding about neuro-inflammatory processes in brain tissue after HI will be, the more closer we will be to developing new treatment strategies for improving outcomes of severe HI.

CONCLUSIONS

1. The IL-6 and IL-10 positive glial cell numbers correlate with the outcome of trauma, as the immediately deceased patients had higher numbers of IL-6 and IL-10 positive cells than in the patients, survived 24 and more hours.
2. The inflammatory reaction in the white substance in the spot of counterstroke was more marked

than in the spot of direct impact, as the number of glial cells that can valuably react to the trauma by producing cytokines, and forms a glial scar as primary damaged glial cells in the spot of the trauma, was higher.

3. The activity of inflammatory reaction depends on the time period after the traumatic event. The most active production of cytokines can be seen in the first minutes and hours after the trauma comparing to remote time periods after the traumatic event.

Conflict of interest: None

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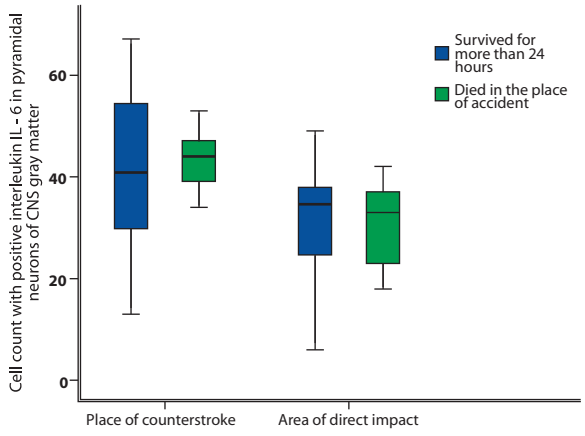

Fig. 1. Number of IL-6 positive pyramidal neurons in the gray substance of the brain in the spots of direct impact and counterstroke.

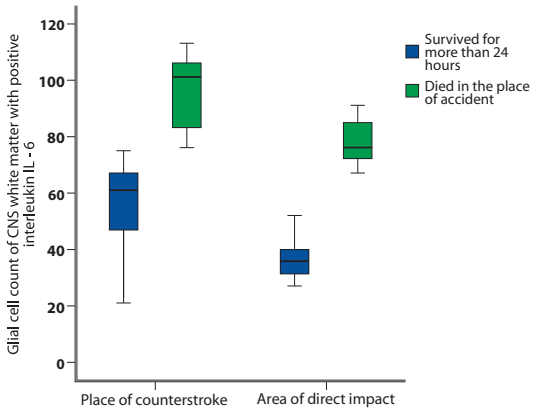

Fig. 2. Number of IL-6 positive glial cells in the white substance of the brain in the spots of direct impact and counterstroke.

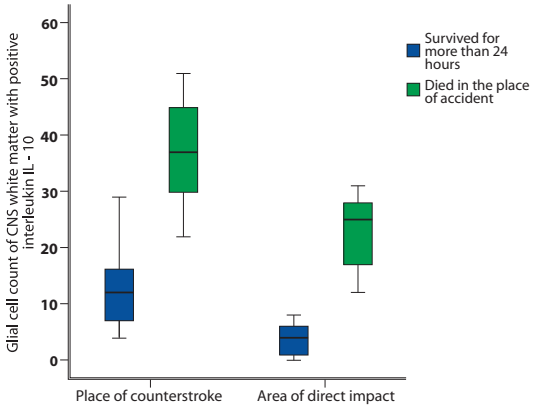

Fig. 3. Number of IL-10 positive glial cells in the white substance of the brain in the spots of direct impact and counterstroke.

Table 1. IL-6 positive pyramidal neuron, glial cell and IL-10 positive glial cell median values, interquartile ranges and statistical significances.

	Survivors				Deceased				
	Min	Max	Median	IQR	Min	Max	Median	IQR	p^1
Counterstroke, cortex IL 6	13	67	41	27,8	34	53	44	10,5	0,89
Direct impact, cortex IL 6	6	49	34,5	17,8	18	42	33	14,5	0,827
p^2	<0,001				0,011				
Counterstroke, white substance IL 6	21	75	61	23,5	76	113	101	25	<0,001
Direct impact, white substance IL 6	8	52	36	9,3	67	91	76	15	<0,001
p^2	<0,001				0,038				
Counterstroke, white substance IL 10	4	33	12	9	22	51	37	18,5	<0,001
Direct impact, white substance IL 10	0	18	4	5	12	31	25	13	<0,001
p^2	<0,001				0,050				

p^1 – Manna Vitnija (Mann – Whitney) test, comparing two independen samples, survivors and deceased
p^2 – Vilkoksona (Wilcoxon) test, comparing two dependent samples, spots of counterstroke and direct impact.

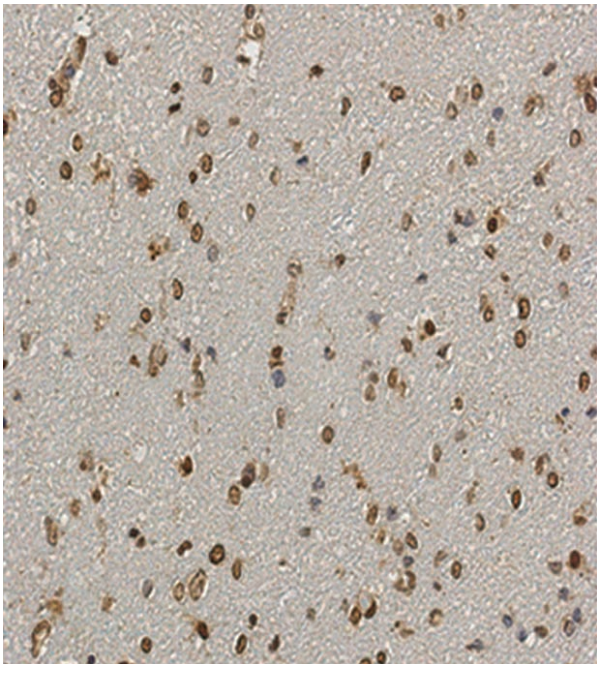

Fig. 4. IL-6 positive glial cells in a white matter of 28 years old man's brain died in the place of accident in injury area, IL-6, X 400. Mouse Monoclonal antibodies against recombinant IL-6 of human origin (IL-6 (NYRhIL6): sc-73319 Mouse Monoclonal antibody, working dilution 1:100, Santa Cruz Biotechnology, USA.

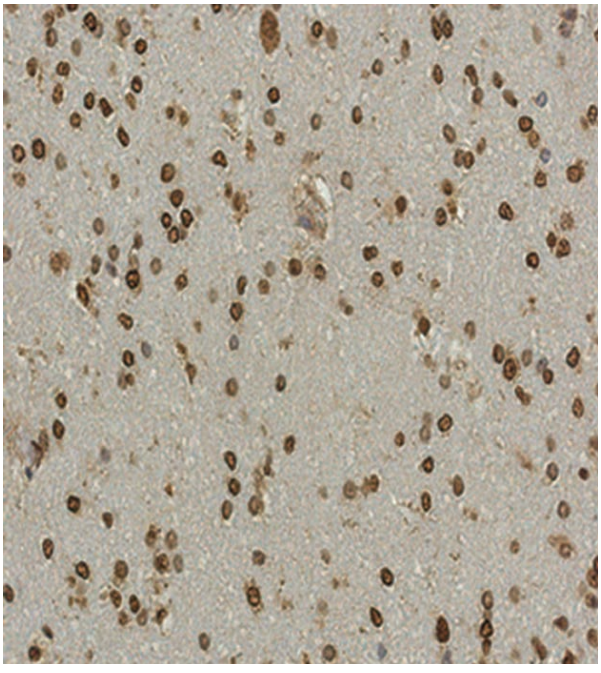

Fig. 5. IL-6 positive glial cells in a white matter of 28 years old man's brain died in the place of accident in the area of counterstroke, IL-6, X 400. Mouse Monoclonal antibodies against recombinant IL-6 of human origin (IL-6 (NYRhIL6): sc-73319 Mouse Monoclonal antibody, working dilution 1:100, Santa Cruz Biotechnology, USA.

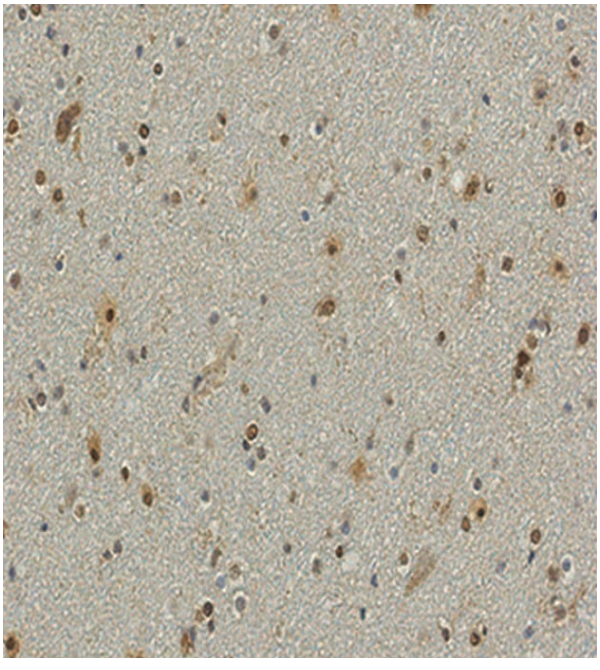

Fig. 6. IL-6 positive glial cells in a white matter of 16 year's old man's brain survived the accident in injury area, IL-6, X 400. Mouse Monoclonal antibodies against recombinant IL-6 of human origin (IL-6 (NYRhIL6): sc-73319 Mouse Monoclonal antibody, working dilution 1:100, Santa Cruz Biotechnology, USA.

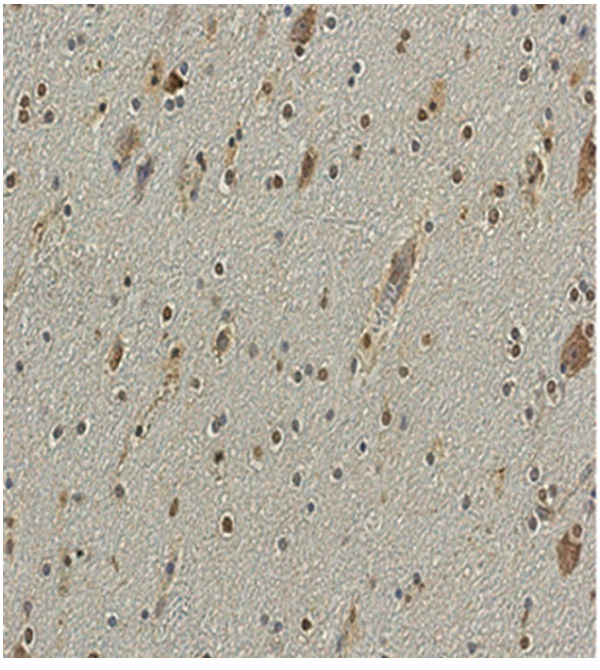

Fig. 7. IL-6 positive glial cells in a white matter of 16 years old man's brain survived the accident in the area of counterstroke, IL-6, X 400. Mouse Monoclonal antibodies against recombinant IL-6 of human origin (IL-6 (NYRhIL6): sc-73319 Mouse Monoclonal antibody, working dilution 1:100, Santa Cruz Biotechnology, USA.

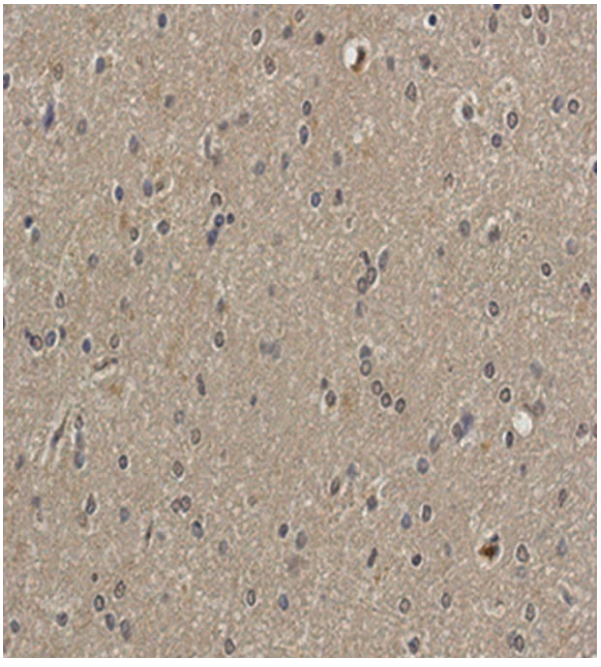

Fig. 8. IL-10 positive glial cells in a white matter of 28 years old man's brain died in the place of accident in injury area, IL-10, 400. Rabbit polyclonal to IL-10, ab34843, working dilution 1:400, Abcam, UK.

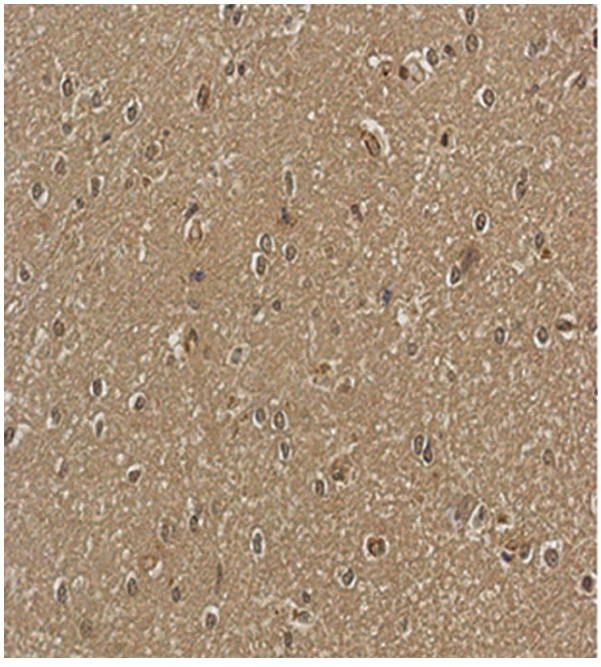

Fig. 9. IL-10 positive glial cells in a white matter of 28 years old man's brain died in the place of accident in the area of counterstroke, IL-10, 400. Rabbit polyclonal to IL-10, ab34843, working dilution 1:400, Abcam, UK.

Fig. 10. IL-10 positive glial cells in a white matter of 16 year's old man's brain survived the accident in injury area, IL-10, 400. Rabbit polyclonal to IL-10, ab34843, working dilution 1:400, Abcam, UK.

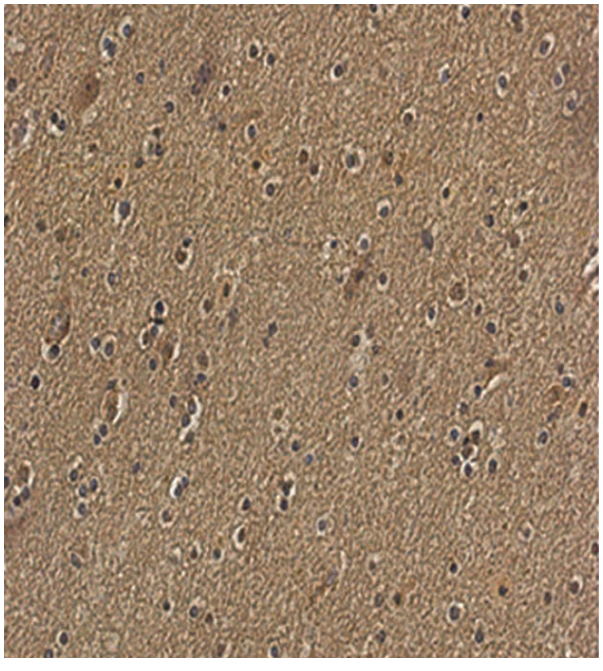

Fig. 11. IL-10 positive glial cells in a white matter of 16 year's old man's brain survived the accident in the area of counterstroke, IL-10, 400. Rabbit polyclonal to IL-10, ab34843, working dilution 1:400, Abcam, UK.

ORIGINAL ARTICLE

Frequency and Localization of Spinal Cord Demyelination in MS Patients, Coexistence of Intervertebral Disc Protrusion

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Summary
Introduction. The importance of spinal cord lesions lies in their presumed greater potential to result in clinical symptoms and disability than white matter lesions in brain, involving as they do clinically eloquent pathways. In clinical practice there are many cases with delayed time of diagnosis in multiple sclerosis patients due to the similarity of the symptoms of disc protrusion to those of MS.
Aim of the Study. To analyze areas of high T2 signal characteristic of demyelination in spinal cord in relationship to clinical manifestation of spinal disturbances and coexistence of intervertebral disc protrusion.
Materials and methods. Retrospective analysis of 150 MS patients was made. Only patients with whole spinal cord scanned and existence of spinal cord lesions in MRI were included. MRI system of GE SIGNA was operated with magnets at field strength 1.0 Tesla, using standardized protocols for spinal cord investigation. Clinical characteristics were analyzed and compared with radiological findings.
Results. Our results showed that spinal cord lesions before clinical manifestation were found in 6.3% of cases. High T2 signal was seen in 88.7% of cervical(C), 58.7% of thoracic (T), and 50% of combination of both(C,T) cords scanned. Higest amount of lesions detected: C3- 12,4%, C2-10,2%, C4-9,8%, C5-9,4% levels. Coexistance of intervertebral disc protrusions in MS patients was observed in 36.5% cases
Conclusions. More frequently lesions were located in cervical rather than thoracic cord, the same as intervertebral disc protrusions. Most of the patients having spinal cord lesions have had at least one clinical episode showing spinal cord dysfunction. Intervertebral disc protrusion were found in about third part of MS patients but with no any statistically visible impact on demyelinating process in the spinal cord.
Key words: multiple sclerosis, demyelination, spinal cord, intervertebral disc, magnetic resonance imaging.
Abbreviations: MS-multiple sclerosis, MRI- magnetic resonance imaging, CD- clinically definite, CIS- clinically isolated syndrome

INTRODUCTION

The clinical and pathological manifestations of multiple sclerosis are due to areas of demyelination which occur throughout the white matter of the central nervous system (8). The distribution of plaques characterizing demyelination is well defined (22): position abutting the ventricles, juxtacortical, subtentorial, spinal cord (1). Clinical presentation of MS is very variable according to localization of lesions. The many of MRI studies show that not all of the affected areas tend to cause disability (10, 15). In about 95-99% of patients with clinically definite MS and in about 50-70% of those at presentation with CIS asymptomatic multiple white matter abnormalities suggestive of demyelination in brain MRI are seen (1,25). Clinically silent cord lesions consistent with demyelination are often detected also in the spinal cord (20). Literature data shows that asymptomatic lesions in spinal cord are verified in about 30-40% of patients presenting with CIS suggestive of MS (4, 9, 16, 23). In CDMS patients lesions in the spinal cord can be detected by MRI in up to 90 % (2, 6, 21). Multiple focal demyelination are usually identified in relapsing remitting (RR) course of disease rather than secondary progressive (SP) or primary progressive (PP) when abnormalities in spinal cord are found to be more confluent, diffuse throughout the cord (3, 10, 14, 15, 17, 21).

The importance of spinal cord lesions lies in their presumed greater potential to result in clinical symptoms and disability than white matter lesions in brain (3, 5, 10, 17), involving as they do clinically eloquent pathways (pyramidal tracts, spinothalamic tracts, and posterior columns) (21).

Typical imaging features of MS lesions in spinal cord are the following: more frequently located in cervical and thoracic portions (7, 8, 13); usually located peripherically (especially in the posterior and lateral columns), but may involve the central gray matter; rarely exceeds two vertebral segments in length and occupy more than half of the cord cross- sectional area; acute lesions may be associated to cord swelling, whereas chronic lesions are not hypointense on T1-weighted images, enhancing lesions are less frequently seen in the spinal cord than in the brain (17), but they are often associated with new clinical symptoms (10,15,28).

Mostly both, brain and spinal cord are affected by demyelination. Nociti et al has found that only 2.3% of patients with CDMS had two or more spinal cord lesions concomitantly with normal brain MRI.

Pathophysiological changes, such as disc protrusion, are surprisingly common findings in cervical spine even among young adults (12, 24), however, some authors have found pathological findings to be more common in symptomatic participants (24). Matsumoto et al., so as Tani et al describes that degenerative changes in spine are highest at C5-6, following C4-5 and C6-7, rare C2-3, C3-4 levels.

AIM OF THE STUDY

The aim of the study was to analyze areas of high T2 signal characteristic of demyelination in spinal cord in relationship to clinical manifestation of spinal disturbances and coexistence of intervertebral disc protrusion.

MATERIALS AND METHODS

Retrospective data of 150 patients (103 female, 47 male) from Latvian MS data base with clinically definite RRMS according to McDonald criteria were analyzed. Only patients with whole spinal cord scanned and existence of spinal cord lesions in MRI were included. MRI system of GE SIGNA was operated with magnets at field strength 1.0 Tesla (5 mm slice thickness), using standardized protocols for spinal cord investigation. Each patient underwent 1 or more serial MRI of the brain and spinal cord but only first imaging of spinal cord with adequate visibility or no artifacts was included. Focal lesions in the spinal cord were defined as areas of hypointensity (T1-weighted images) or hyper-intensity (T2-weighted images) sharply demarcated from surrounding tissue. Clinical characteristics (relapse type by localization and amount, onset of disease) were analyzed and compared with radiological (number and distribution of spinal cord lesions) findings. Regarding protrusions of intervertebral discs only quantity, distribution and correlation with cord lesions were examined. No gradation by severity of intervertebral disc protrusion was made.

Descriptive analysis was performed using statistical program SPSS 17. To reflect the standard deviations for the calculated values standard error of the mean was used. Correlations were calculated as Spearman's rank correlation coefficient. For correlation coefficients a *P* value of less than 0.01 was considered statistically significant.

RESULTS

All together 701 (from total of 150 patients) demyelinated loci of spinal cord were analyzed on the MR images (Table 1). Total of 54.1% of patients experienced not more than 4 lesions.

Table 1.Total and average amount of lesions

	Amount of lesions	Average amount of lesions/patient
Total	701	9,4
Cervical	434	5,86
Thoracic	267	3,58

Total of 33.5% patients experienced spinal symptoms during the first manifestation of MS. Age at onset of disease varied from 13-53 years; median parameters are seen in Table2. The mean age at onset of those patients who initially presented with cerebral manifestation was 32.02±9.41 years in total, 32.06±9.91 in women, and 30.2±8.57 in men, in cases of spinal manifestation 31.02±10.9 in women and 29.52±6.1years in men. Most of the patients have had at least one spinal episode before MRI scan (average 1.14±0.84) with duration ranged from <1 month to more than 10 years. The patients having MRI performed 5-10 years and more since onset of cord symptoms were describing at least 3 spinal episodes.

Table 2.Summary of clinical and radiological characteristics

Median age at onset of MS(total)	31.05±9.15 years
Median age at onset of spinal manifestation	30.37±9.7 years
Duration of spinal MS presentation at the time of MRI examination	25.2%- <6 months, 10.8%- 6-12 months, 14,4% - >10 years
Spinal cord lesions before clinical manifestation	6.3% of cases
Episodes of spinal disturbances pre MRI.	42.6%-1, 24.3%-2 5.4%-3
Duration to MRI if only one clinical episode of spinal manifestation noted	<6 months 33.4%, <1 yr 46.1%, >5yr 26.9%.
MRI duration >1yr and at least 2 spinal episodes	48.5%
MRI duration >10 yr and only 1 spinal relapse	4 cases
High T2 signal	88.7%-Cervical 58.7%- Thoracic 50%- Cervical+Thoracic
Amount of lesions having 1 spinal relapse	49.2% 2-4 loci, 11.1%- >8 loci, Average-4.9 loci/patient

The correlation between age at onset and amount of spinal lesions was not fixed (p=0.025). There was no significant difference between increased number of lesions in both groups: cerebral onset or spinal onset (p=0.130).

Concerning amount of lesions in spinal cord showed correlation between increased lesion count and higher relapse rate (p=0.008), which is summarized in Table3.

Table 3. Correlation between average amount of lesions and relapse rate.

N spinal relapse	Amount of lesions	
	Mean	95%CI
0	3.9±2.5	2.99-4.82
1	4.6±2.8	3.89-5.31
2	5.48±3.02	4.44-6.52
3	5.7±3.2	3.04-8.45

In all 150 cases analyzed total number of focal loci varied from 1 to 16. Only one lesion was detected in 8.3%, 2-13%, 3-10.7%, 4-15.4%, 7-10.7%, and 8-16 in 12% of patients. Silent lesions were more visible in cervical proportions (31 of 144 patients) compare to thoracic (10 of 144 patients), Fig.1.

Amount of lesions according to relapse rate

	Amount of spinal relapse / Amount of lesions	1	2	3	4	5	6	7	8	9	10	11	13	>15	
Clesions	0	7	7	6	6	1	2	2							31
	1	9	14	14	11	3	1	4							56
	>1-2	5	4	5	10	9	1	3							37
	>=3	1	0	2	3	0	1	1							8
TNesions	0	0	7	0	1	0	0	0	1	1					10
	1	5	16	9	4	2	3	0	0	1					40
	>1-2	3	17	4	2	2	2	0	0	1	0	0	1		32
	>=3	2	2	1	0	1									6
Total	0	5	5	5	7	3	3	2							30
	1	5	11	11	9	7	3	9	4	1	1	1		1	63
	>1-2	3	5	1	9	5	5	7	1	2	3	1		1	43
	>=3	1	0	0	1	4	0	0	0	1	1	0			8
															144

Fig. 1.

Data regarding distribution of lesions in spinal cord by MRI is showed in Fig.2.

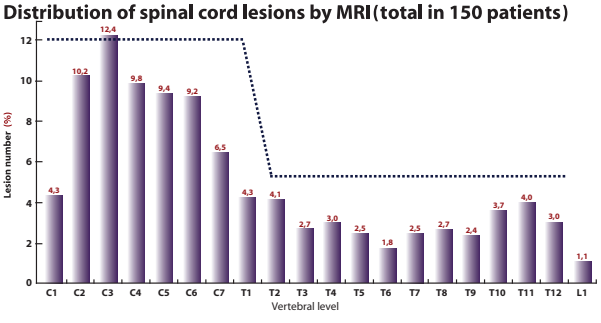

Fig. 2

Coexistance of intervertebral disc protrusions in MS patients was observed in 36.5% cases; with mean number 2.07±1.17. The distribution of protrusions is presented in

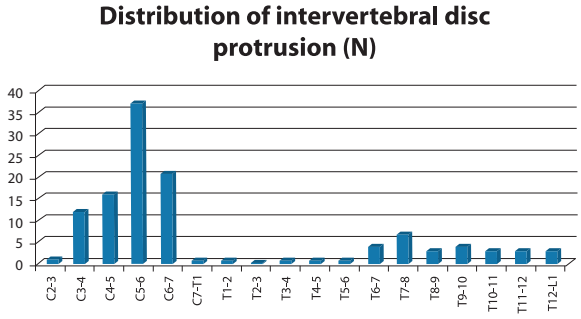

Fig. 3.

No correlation between coexistence of intervertebral disc and spinal cord lesions at the same level was found (p=0.577).

DISCUSSION

There are no many literature data describing spinal cord abnormalities, as well as degenerative findings of spine in MS patients. The most studies are performed in late 90ties. But with increasing quality of MRI techniques over the last years this question regarding evaluation of spinal cord has became more actual and more studied. Based on our results and on previous studies, the MRI characteristics of spinal cord in patients suffering from MS include well defined distribution of lesions and correlation with clinical data. The both, previous information and our data shows that demyelinating lesions may be clinically silent, and also that frequency of lesions located in spinal cord is high. The distribution of spinal cord lesions which is summarized in Fig.2 is quite similar to that reported in previous trials. The same similarities are found if compare with other authors regarding localization of degenerative changes in spine. We could not found reports where characteristics of intervertebral disc abnormalities were described based on MS population that why our results were compared with data from general population. So as there are cases described when MS is not initially diagnosed because of similarity of the symptoms of disc protrusion to those of MS and the same conversely, it would be very interestingly to appraise also correlations with clinical findings in both situations. The correlations obtained between spinal relapse rate and increased amount of spinal cord lesions demonstrate necessity for enlarged evaluation of clinical signs in MS patients, especially of those when MRI of spinal cord is not performed and clinical symptoms are contributing to anatomical localization typical to those which are showed as more frequently involved in demyelinating process. Also possibility of coexistence of degenerative changes should be kept in mind especially in cases when older patients are examined so as the findings are proportionally turning up with age. Although no correlation detected between level of intervertebral disc protrusion and location of spinal cord lesions, interestingly that both are more frequently found in cervical proportion, around C3 to C7 level. We are not able to rule out that the potential investments in producing similar studies with larger population may result with new surprising findings.

CONCLUSIONS

More frequently lesions were located in cervical rather than thoracic cord. Most of the patients having spinal cord lesions have had at least one clinical episode showing spinal cord dysfunction. No correlation between spinal cord lesions and coexistence of intervertebral disc protrusion at the same level were found.

Conflict of interest: None

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ORIGINAL ARTICLE

Plasma Fibrinogen Level and Postoperative Bleeding after On-pump Cardiac Surgery

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Summary
Introduction. Fibrinogen plays a critical role in achieving and maintaining haemostasis, particularly in patients undergoing cardiac surgery where significant blood loss can be anticipated.
Aim of the study. Identify plasma fibrinogen level in different time points and its association with postoperative bleeding after on-pump cardiac surgery.
Materials and methods. A total of 135 adult cardiac surgical patients (mean age 67±9, 53% men) were enrolled in prospective study at Pauls Stradins Clinical University Hospital between December 2009 and August 2010. Blood samples testing for plasma fibrinogen level, prothrombin time (PT), platelet count (PLT), haemoglobin (Hb) were collected at day before surgery (T0), at admission in intensive care unit (T1), 6 and 24 hours after operation (T6, T24). Postoperative bleeding volume during first 24 hours was registered. To allow compare patients excessive bleeding was defined as 24-hour blood loss of > 500 ml. Two patients groups were made: I group ≤ 500ml/24h and II group > 500ml/24h blood loss.
Results. Eleven patients (8,1%) had a surgical bleeding. Bleeding > 500ml/24h because of haemostatic disorders post cardiopulmonary bypass was observed in 56 out of 124 patients (45%). Postoperative bleeding volume significantly correlated with plasma fibrinogen concentration at all time points. The strongest inverse correlation was found between preoperative fibrinogen level and postoperative blood amount ($r = -0.35$, $p < 0.001$). Fibrinogen level at all time points significantly differed between I and II group ($p = 0.004$, $p = 0.003$, $p = 0.006$, $p = 0.002$). The strongest association between fibrinogen level and other coagulation parameters showed fibrinogen and PLT count at T1 ($r = 0.45$, $p < 0.001$). Correlation in lesser degree was found between fibrinogen and PT and Hb concentration before operation.
Conclusions. Plasma fibrinogen level at different time points could be as a possible predictor of an increased bleeding risk and may provide information about bleeding volume after cardiac surgery. The results show fibrinogen interaction with PLT count and its role in haemostasis.
Key words: Fibrinogen, haemostasis, heart surgery, bleeding.

INTRODUCTION

Complex cardiac surgery is frequently accompanied by excessive perioperative bleeding resulting from coagulation system impairment, inadequate surgical haemostasis, or both (15). Bleeding continues to be the first cause for early mediastinal re-exploration after open heart surgery. Suggested pathomechanisms include platelet dysfunction, hyperfibrinolysis and decreased clotting factor activities due to haemodilution, activation and increased consumtion (11). Excessive bleeding may result in patients receiving massive blood transfusions or suffering of life-threating complications such as myocardial infarction, low cardiac output syndrome, respiratory failure and pneumonia, severe arrhythmia, deep sternal wound infections, hepatic and renal insufficiency and need for haemofiltration, cardiac tamponade and, consequently, associated increased mortality (1,7).

New data suggest that fibrinogen plays a critical role in achieving and maintaining haemostasis, particularly in patients undergoing cardiac surgery where significant blood loss can be anticipated (8,17). Identify patients fibrinogen level before and after surgery increases our ability to predict bleeding and offers the possibility of early and targeted coagulation therapy.

Fibrinogen is a key protein in the human coagulation system and, thus, a potential biomarker for excessive postoperative bleeding (14). Also known as clotting factor I (one), in healthy adults its normal concentration in plasma is 2-4,5 g/l (8) but in cardiac surgery patients fibrinogen level in the lower normal range may be too low to ensure an appropriate coagulation because the haemostatic system is challenged by the use of cardiopulmonary bypass (CPB). Fibrinogen is converted in plasma by thrombin into a fibrin clot at the site of tissue damage to minimize blood loss and initiate tissue repair (14,18). In primary haemostasis fibrinogen supports platelet aggregation with formation of a platelet plug, and in secondary haemostasis the formation of an insoluble fibrin clot (2). Dilution coagulopathy and platelet consumption have been suggested to be two important factors in the pathogenesis of bleeding after cardiac surgery (4,11,16). In experimental studies fibrinogen counteracts dilution coagulopathy and impaired haemostasis caused by thrombocytopenia and possibly because of these effects reduces bleeding in cardiac surgery patients.

Fresh frozen plasma can be used to raise plasma fibrinogen concentration but it can not increase fibrinogen level enough to create a strong, stable clot and it may also cause volume overload. One of the main source of fibrinogen is Cryoprecipitate. Infusion of fibrinogen concentrate also has been used to treat severe surgical and trauma related bleeding (5) and to substitute hereditary fibrinogen deficiency disorders (12). Preoperative fibrinogen concentrate infusion also reduces bleeding after coronary artery bypass grafting (CABG) (10). However efficacy, dose-responsiveness and potential side effects are little studied in humans.

AIM OF THE STUDY

Identify plasma fibrinogen level in different time points and its association with postoperative bleeding after on-pump cardiac surgery.

MATERIALS AND METHODS

Patients

After obtaining ethics committee approval of the Pauls Stradins Clinical University Hospital and written informed patient consent, initially 135 adult cardiac surgical patients (mean age 67±9, 53% men) were enrolled in prospective, observational study between December 2009 and August 2010. Inclusion criteria were age 18 to 70 years, first-time coronary artery bypass grafting or/and valve replacement surgery in CPB, baseline coagulation tests in normal values and at least 5 days patient did not receive any anticoagulants, antiagregants and non-steroidal anti-inflammatory drugs excluding platelet dysfunction. The last dose of low-molekular-weight heparin was administered the evening before surgery.

Our exclusion criteria were emergency and redo operations, preoperatively hemostatic disorders with a history of haemorrhagic events or chronic coagulopathy (PT below 50%, INR greater than 1,5, fibrinogen level below 1,5 g/l, platelet count lower than 100 x 10^9/l), severe renal or/and hepatic dysfunction.

Perioperative management

All patients underwent standardized opoid-based anesthesia and surgical techniques. The anesthesia was induced with fenthanil, midazolam, propofol and cisatracurium and maintained with sevoflurane. During CPB, anesthesia was maintained with propofol. Patients did not received antifibrinolytic drugs. Before the start of CPB heparin was administered in a dose 300 to 400 units/kg initially and 5,000 to 10,000 units as indicated were given to achieve and maintain a target activated coagulation time (ACT) above 480 seconds before and during CPB.

Standart nonpulsatile CPB technique with moderate hypothermia (bladder temperature 34-35^{0}C) and haemoduliton was used. Weaning of CPB was performed after rewarming to a bladder temperature of at least 36^{0}C. After separation from CPB, protamine was administered in a ratio of 1 mg:100 units of heparin, additional protamine was administered until ACT had returned to baseline – less than 130 seconds.

Study design and analyses

The association between bleeding and the following pre- and postoperative variables was investigated: age, sex, body mass index (BMI), ejection fraction, type of surgery, CPB duration, aorta occlusion time, reperfusion time, anticoagulation therapy. Association between bleeding and plasma fibrinogen level was analyzed in different time points as well as fibrinogen correlation with Hb, platelet (PLT) count and prothrombin time (PT).

Blood samples testing for fibrinogen, Hb concentration, PT and PLT count were collected and analyzed at day before surgery (T0), at admission in intensive care unit (ICU) immediately after operation (T1), 6 hours and 24 hours after surgery (T6, T24).

Plasma fibrinogen concentration was determined according to the method by Clauss where citrated plasma is brought to coagulation by a large excess of thrombin (Multifibren U reagent, Siemens Healthcare Diagnostics, U.S.A.) The reference value is 1,8-3,5 g per L. PT was analyzed with a prothrombin complex assay (Lyophilized Dade®Innovin® reagent, Siemens Healthcare Diagnostics, U.S.A.).

Hb and PLT count were analyzed using Sysmex CA 1500. Hb concentration and PLT count were determined by Beckman Coulter LH 750 Hematology Analyzer. The Coulter LH 750 uses impedance technology to measure PLT count and haemiglobincyanide method to measure Hb concentration.

Postoperative bleeding volume from chest tube drainage (CTD) were recorded at 24 hour postoperatively as milliliters. To allow comparison between patients excessive bleeding was definated as more than 500 ml in 24 hours. Two groups of patients were made according to blood loss: I group ≤ 500ml/24h (n = 68) and II group > 500ml/24h (n = 56). In case of reexploration, bleeding volume until and after reexploration at 24 hour was registered. A surgical bleeding was diagnosed at the time of reexploration if a specific site/sites was identified. Patients with surgical bleeding were excluded from further study. If reason of bleeding was not diagnosed during reexploration we registered it as bleeding of haemostatic disorders.

Statistical analysis

All statistical analysis was performed using Statistical Package for the Social Sciences (SPSS®16.0). Continuous variables were described as mean standard deviation (SD) and categorical variables as percentages (%). Statistical significance was defined as a p value of less than 0.05. The sample size was determinated by the number of cases at the clinic during the study period. Linear regression as Pearson's correlation coefficient was used to analyse the relationship between hematologic and demographic, surgical data and the volume of postoperative bleeding. The association of fibrinogen and haemostasis screening tests with the amount of drainage blood at T24 was examined by multiple linear regression analysis. Comparison between two patient's groups were done with two-sample t tests for continuous data and with chi-square tests for categorical data.

RESULTS

Clinical course

One hundred thirty five adult cardiac surgical patients were included in the study. There were no in-hospital deaths. Three patients had following postoperative complications: one patient – nosocomial pneumonia, second - hepatorenal syndrome and third - mediastinitis. Fourteen patients had reexploration because of excessive bleeding or pericardial tamponade 10 minutes till 145 hours after surgery. A surgical bleeding was diagnosed at the time of reexploration in eleven (8.1%) patients out of fourteen patients. Bleeding reason was not found in 3 patients and it was registered as bleeding of haemostatic disorders. These 11 patients were not included in future study.

Excessive bleeding defined as 24-hour blood loss of more than 500 ml post CPB was observed in 56 (group II) out of 124 patients (45%). In relation to the variations in the mean ages, genders, BMI, ejection fraction and surgical parameters of the patients in the two groups, there was no significant difference. Significantly differed 24 hour blood loss between groups. Patient characteristics are presented in Table 1.

Preoperatively 110 patients were treated with one or more anticoagulants. Eighty-eight patients received aspirin within 6 ± 4 days before surgery, clopidogrel – 28 patients (8 ± 4 days), low-molecular-weight heparin – 76 patients, the last injection given 12 hours before surgery. Four patients was treated with warfarin within 5 ± 2 days before operation. There was no statistically significant difference in blood loss between patients with various anticoagulants.

Fibrinogen

All patients had a preoperative fibrinogen concentration above the lower limit of 1.8 g per L. The mean fibrinogen concentration at T0 was 4.7±1.5 g per L (range 1.8-10 g per L). A total of 42 patients (34%) had concentrations within the normal range and the larger part of patients 82 (66%) had higher concentrations > 3,5 g per L. At T1 mean fibrinogen level was 3.29±0.9 g per L, at T6 was 3.5 ± 0.9 g per L, at T24 was 4.1± 1.03 g per L. The highest decrease of fibrinogen concentration was between T0 and T1 time points respectively by 30 %.

Comparing fibrinogen concentration between II (n = 56) and I group (n = 68) it significantly differed at all time points particularly at T1 and T24 (Figure 3): at T0 - 4 *vs*. 4.8, p = 0.004; at T1 - 3 *vs*. 3.5, p = 0.003; at T6 - 3.3 *vs*. 3.7, p = 0.006; at T24 - 3.8 *vs*. 4.4, p = 0.002.

Association between pre- and postoperative variables and bleeding

Neither demographic and surgical parameters correlated to 24 hours blood amount.

Strongest inverse correlation with postoperative bleeding showed fibrinogen level preoperatively ($r = -0.35$, $p < 0.001$; Figure 1) and 24 hours after surgery ($r = -0.3$, $p < 0.001$). Fibrinogen level at T1 and T6 time points showed correlation with postoperative blood amount as well but this correlation was considerably less pronounced ($r = -0.26$, $p = 0.01$; $r = -0.2$, $p = 0.02$).

Analyzing fibrinogen association with other coagulation parameters, we found statistically significant correlation between fibrinogen level and PLT count preoperatively and at all postoperative time points: at T0 $r = 0.34$, $p < 0.001$; at T1 time point we observed the strongest correlation (Figure 2) $r = 0.45$, $p < 0.001$; at T6 $r = 0.4$, $p < 0.001$; at T24 $r = 0.43$, $p < 0.001$. As well as between fibrinogen level and PT at T0, T1 and T6 time points: at T0 $r = 0.24$, $p = 0.008$; at T1 $r = 0.35$, $p < 0.001$; at T6 $r = 0.31$, $p = 0.001$. Fibrinogen level and Hb concentration showed correlation at T0 $r = -0.3$, $p = 0.002$.

DISCUSSION

The main finding on this study is that fibrinogen plasma concentration significantly differed between groups and correlated with postoperative blood amount as well indicating that fibrinogen could be as predictor of postoperative bleeding. Although all patients had preoperative fibrinogen levels above the normal lower limit it could suggest that fibrinogen level in the lower normal range may be too low to ensure normal coagulation when the haemostatic system is challenged by cardiac surgery and the use of CPB. *Blome* with coauthors confirms that in their study also all patients had fibrinogen level within the normal reference range or elevated, but higher blood loss was significantly associated with lower fibrinogen levels (3).

Cardiac surgery and the use of CPB induce substantial alterations in the hemostatic system (16). The role of fibrinogen level as a predictor of postoperative bleeding has been investigated in a few studies with conflicting results (3,8,13,20). *Blome* with coauthors (3) found pre- and postoperative fibrinogen correlation with drainage blood volume. *Wahba* at al. (20) reported significant correlation as well including eighty-nine patients undergoing CABG surgery. *Karlsson* et all. (9) obtained similar results in their study of 170 patients, concluded that measurement of preoperative fibrinogen provides information about bleeding volume and also transfusion requirements. They found correlation between postoperative bleeding volume and preoperative fibrinogen concentration ($r = -0.53$, $p<0.001$) and it was an independent predictor of postoperative bleeding volume. Whereas *Lui* with coauthors were not able to show any association (13).

In a few studies has previously been shown differences in fibrinogen levels between bleeders and nonbleeders (6,8,20). In this study we found statistically significant difference between our two patients groups as well particularly at admission in ITC and 24-hour after surgery. On arrival in the operating theater fibrinogen concentration could be affected by perioperative fluid replacement and therefore at this time point fibrinogen level possible has not so important role as predictor of expecting blood amount. Preoperatively measured plasma fibrinogen could be with the highest predictive role where we found significant difference between both patient's groups as well. A large study in 894 patients observed a significant association between post-bypass screening tests of haemosthasis, consisting of PLT count, PT, activated partial thromboplastin time, fibrinogen

and fibrin degradation products and 16-hour CTD (6). Of these parameters only post-bypass fibrinogen differed significantly between bleeders and nonbleeders. *Jimenez Rivera* et al. (8) found that fibrinogen level significantly differed between bleeders and non-bleeders either.

Regarding to fibrinogen association with other coagulation parameters, using simple linear regression analysis, the strongest correlation was found between fibrinogen and PTC count at admission in ICU. In experimental studies, fibrinogen counteracts impaired hemostasis caused by trombocytopenia (19) because of fibrinogen role in primary and secondary hemostasis (2). It is possible that these effects contribute to the reduce bleeding in patients with higher fibrinogen levels and PLT count concentration.

CONCLUSIONS

Plasma fibrinogen level at different time points could be as a possible predictor of an increased bleeding risk and may provide information about bleeding volume after cardiac surgery. The results show fibrinogen interaction with PLT count and its role in haemostasis.

Conflict of interest: None

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Table 1. Characteristics of all patients (total) and of the group with blood loss in 24h > and ≤ 500ml

Variables	Total (n=124)	Group II (n=56)	Group I (n=68)
Demographic			
Sex (% male)	66 (53.2%)	30 (53%)	36 (53%)
Age (years)	66.7±9	67±9	66±9
BMI (kg/m²)	28.6±5.2	27±0.5	29±5.8
EF (%)	55.5±7.2	55±7.8	56±7
Surgical parameters			
CABG, n (%)	48 (39%)	22 (39.3%)	26 (38.2%)
Valve replacement, n (%)	46 (37%)	20 (35.7%)	26 (38.2%)
Combined surgery, n (%)	30 (24%)	14 (25%)	16 (23.6%)
CPB duration (min)	104±35	104±35.5	104±35.8
Aorta occlusion time (min)	65.7±24	65±24	66±25
Reperfusion time (min)	32.9±14	33.7±13	32±15
Preoperative medications			
Aspirin, n (%)	88 (71%)	35 (39.3%)	53 (78%)
LMWH, n (%)	76 (61.3%)	40 (71.4%)	36 (53%)
Clopidogrel, n (%)	28 (22.5%)	8 (14%)	20 (29%)
Warfarin, n (%)	4 (3.2%)	3 (5.3%)	1 (1.5%)
Blood loss (ml/24h)	543±268	768±236*	357±92*

Data are reported as mean ± SD or number (%), * $p < 0.05$
Abbreviations: BMI – body mass index, EF – ejection fraction, CABG – coronary artery bypass grafting, CPB – cardiopulmonary bypass, LMWH – low-molekular-weight heparin

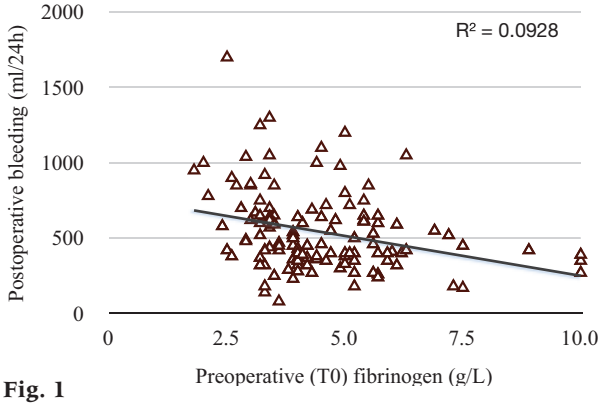

Fig. 1

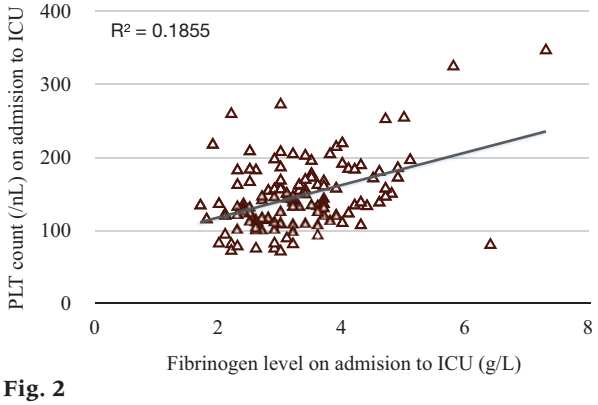

Fig. 2

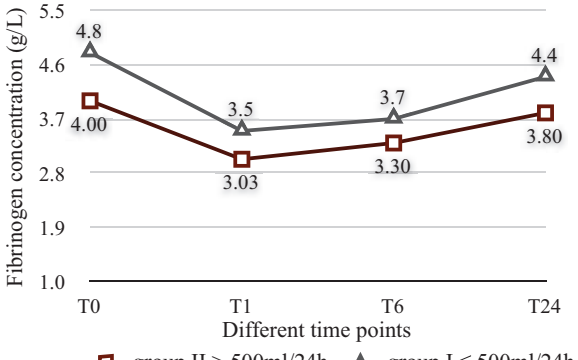

Fig. 3

ORIGINAL ARTICLE

Results of Cardiopulmonary Resuscitation during In-Hospital Cardiac Arrest

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Summary
Introduction. The performance of cardiopulmonary resuscitation (CPR) has an important position in the chain of survival, but despite new techniques and technology the survival rates from cardiac arrest are still low. Regular CPR analysis has not been carried out in Latvia.
Aim of the study. To evaluate results of CPR in the Pauls Stradins Clinical University Hospital during in-hospital cardiac arrest.
Materials and methods. The study was conducted in the Pauls Stradins Clinical University Hospital during the time period between January, 2010 and March, 2011. There were 221 adult patients with an established and recorded cardiac arrest and performed CPR included in the retrospective research. The information was analyzed by medical records. The obtained results were expressed in percents and compared, using the Pearson's Chi-square (Pearson $\chi 2$) test.
Results. The return of spontaneous circulation (ROSC) was more commonly achieved at the cardiological intensive care units (58%). The most commonly used algorithm that initiates the CPR was pulseless electrical activities /asystole – used in 73% of cases. Most frequently the ROSC was achieved in the cases of ventricular fibrillation/pulseless ventricular tachycardia, but the largest number of unsuccessful episodes of CPR was observed in the cases of pulseless electrical activities/ asystole.
Conclusion. Results of CPR were different in intensive care settings. ROSC is influenced by initial heart rhythm. The cardiac arrest time did not influence the efficiency of cardiopulmonary resuscitation.
Key words: cardiac arrest; in-hospital cardiopulmonary resuscitation; return of spontaneous circulation.

INTRODUCTION

Cardiopulmonary resuscitation (CPR) is one of the most important emergency actions in life-threatining conditions. The performance of cardiopulmonary resuscitation has an important position in the chain of survival (17), but despite new techniques and technology the survival rates from cardiac arrest are still low (7; 4; 16) and the incidence of in-hospital cardiac arrest is rarely reported in the literature (17). Cardiopulmonary resuscitation is a complex of emergency procedures that, if performed correctly, can provide the necessary minimum of circulation until return of spontaneous circulation (ROSC) to a patient with a sudden (unexpected) cardiac arrest (6). Both out-of-hospital and in-hospital cardiopulmonary resuscitation is performed according to the Guidelines for Resuscitation 2010 adopted by the European Resuscitation Council (ERC) and updated once in five years. The ERC Guidelines 2010 emphasize the quality of cardiopulmonary resuscitation and the post-resuscitation care as the big challenge for the next five years. The efficiency of CPR in decreasing the death-rate from avertable causes is a very important factor. However there are relatively few data available on the efficiency of cardiopulmonary resuscitation at hospitals, which play an essential role in the chain of survival, but in Latvia such systematic studies are not conducted at all. Despite the fact that the cardiopulmonary resuscitation was introduced more than 50 years ago, the survival rate is still relatively low (1) and there is no trend of improvement of the situation observed, although the technologies have developed rapidly (11). Therefore it is important to analyze whether the purchased technologies are applied to the full extent in provision of the CPR process and the quality control. As the factors, determining the efficiency of the in-hospital CPR, several authors mention the importance of the patient's location at the moment of cardiac arrest in a monitored or non-monitored area (1, 10), the pathogenetic mechanism of the cardiac arrest (ventricular fibrillation/ pulseless ventricular tachycardia, pulseless electrical activity, asystole (12, 10), the time of day when the cardiac arrest has occurred (5, 14, 3), implementation or improvement of training for medical persons, implementing of the resuscitation protocols in hospitals, the quality control of CPR during its performing (9), but the obtained data are incomplete and contradictory. Reported survival to hospital discharge varies from 0% to 42%, the most common range being between 15% and 20% (17). Therefore the problem requires an additional study. It is worth mentioning that in the majority of cases it is possible to assess the quality of CPR only indirectly, according to the manikin studies in the process of training or according to the experimentally obtained data. The problem of the in-hospital CPR results is urgent all over the world (5, 15), therefore we should continue analyzing the results and efficiency of CPR both out-of-hospital and in-hospital to realize the current situation and look for ways to improve the situation in the future. This research will study the results of cardiopulmonary resuscitation during in-hospital cardiac arrest.

AIM OF THE STUDY

The aim of this study was to evaluate the results of CPR in the Pauls Stradins Clinical University Hospital during in-hospital cardiac arrest.

MATERIALS AND METHODS

The study was conducted in the Pauls Stradins Clinical University Hospital during the time period between January, 2010 and March, 2011. There were 331 adult patients with an established and recorded cardiac arrest and performed CPR included in the retrospective research. 236 patients were treated in intensive care setting in the mode of continuous observation with monitors and medical staff on duty: 137 patients in general intensive care unit (ICU), 50 patients in the cardiological ICU and 49 patients in the Emergency department (ED). The ICU beds were inspected and it was established that they were similarly equipped with monitors, providing continuous registration of the vitally important parameters: the heart rhythm and heart frequency, the non-invasive arterial blood pressure and the level of oxygen saturation in blood. An indication for admitting patients in the intensive care setting was haemodinamically unstable condition, a necessity for intensive treatment and the risk of cardiac arrest from avertable causes. 95 patients were treated in hospital general ward setting, because their condition was recognized as stable, therefore no continued monitoring was indicated. In the general wards the staff had only basic life support (BLS) skills and defibrillation was no immediately available. There were 110 patients excluded from the study: CPR was started outside Pauls Stradins Clinical Hospital in 4 cases, the brain death was established in 9 cases, in 9 cases the *ex consilio* decision regarding symptomatic treatment was taken, in 88 cases the records on performing the CPR were too incomplete for further analysis.

The further analysis was conducted on 221 patients. There were 252 cardiopulmonary resuscitation episodes analyzed in total. 31 patients received a CPR more than one episode. It was considered that the CPR is effective if return of spontaneous circulation is established, which remains for 20 minutes and longer (a short-term ROSC), including discharging from the hospital (a long-term ROSC). The cardiopulmonary resuscitation results were analyzed in the general ICU, where the patients of various pathologies were concentrated, in the cardiological ICU, where cardiological patients mostly were concentrated, and in the Emergency department, where were various patients in life-threatening condition at the time of hospital admittance. Besides in all cases the impact of pathogenetic mechanism of cardiac arrest on the CPR result and the impact of the time of day on the CPR result were analyzed. The following cases were considered, analyzing the dependence of the CPR results on the cardiac arrest: ventricular fibrillation/ pulseless ventricular tachycardia – VF/VT (n=58), the pulseless electrical activity/ asystole - BEA/A (n=186) and the indeterminate rhythm (n=8) – in cases, when the initial rhythm was not provided.

Analyzing the cardiopulmonary resuscitation results depending on the time of the day, the 12 – hour intervals, 06:00 – 18:00 (day) and 18:00 – 06:00 (night), were selected due to different number of the personnel resources. There was reducing of medical staff, which can perform the CPR, at night.

The obtained results were expressed in percents and compared, using the Pearson's Chi-square (Pearson $\chi 2$) test. The statistically valid p value was <0, 05.

The information on the efficiency of CPR in the hospital general ward setting, where the patients were not monitored, was summarized. The cardiac arrest was established for 95 patients in this category, but the cardiopulmonary resuscitation was started on 7 patients.

RESULTS

There were 221 adult patients from the age of 23 to 92 years and 252 CPR episodes included in the study, seeing that the cardiopulmonary resuscitation on 31 patients was performed more than one episode. 48% of the patients were females (n=106) and 52% were males (n= 115). The average age of females was 68 years; the average age for males was 65 years.

As it can be seen in Figure 1, the CPR was most frequently performed on patients from the age of 50 to 89.

The most frequent basic disease for all age groups was of cardiac origin (n=101; 46%), the second place was taken by the diseases of neurological origin (n=51; 23%). As it can be seen in Figure 2, other groups of diseases, excluding the diseases of oncological origin (n=22; 10%) and surgical origin (n=19; 8%), are significantly smaller.

In the general ICU, the cardiac arrest was occurred and the CPR started in 214 cases (Table 1): on 127 patients in the general ICU, on 46 patients in the cardiological ICU and 41 patients in the Emergency departament. Most frequently the ROSC was achieved in cardiological ICU– 58% (n=35), the most rarely in the Emergency department – 15% (n=9). The ROSC was established for 60 patients out of 214 patients placed in the monitored area. Using the parametric Pearson's chi-square (Pearson $\chi 2$) test, the value p was obtained, which indicated that the differences of the ROSC frequency among the departments were statistically valid (Chi2 $p<0.0001$).

Analyzing the dependence of the ROSC frequency on the cardiac arrest mechanism (Table 2), the obtained data provide evidence that the initial heart rhythm in 73% of episodes (n=186) was a pulseless electrical activity/ asystole (BEA/A), in 23% of episodes (n=58) – ventricular fibrillation/ pulseless ventricular tachycardia (VF/VT) and in 4% of episodes (n=8) the initial heart rhythm was not determinate. Most frequently the ROSC was achieved in the cases of ventricular fibrillation/ pulseless ventricular tachycardia, but the largest number of unsuccessful episodes of CPR was observed in the cases of pulseless electrical activities/ asystole. Using the parametric Pearson's chi-square (Pearson $\chi 2$) test, the value p was obtained, which indicated that the differences of ROSC frequency among the blood circulation mechanisms were statistically valid (Chi2 $p<0.0001$).

Analyzing the dependence of the ROSC frequency on the time of day (Table 3), it was established that 143 episodes of the cardiac arrest and CPR were registered in the daylight from 06:00-18:00, but 190 episodes were registered in the evening and night hours. Most frequently ROSC was achieved in the time interval from 06:00 – 18:00. Using the parametric Pearson's chi-square (Pearson $\chi 2$) test, the value p=0.059 was obtained, which indicated that the differences of the ROSC frequency between different times of the day were not statistically valid.

Analyzing the CPR results in the group of non-monitored patients, the data was obtained that ROSC was achieved in five cases out of seven. Due to too different number of CPR cases in the groups of monitored patients (n=214; ROSC for 60 patients) and non-monitored patients (n=7; ROSC for 5 patients), the difference between the ROSC for monitored and non-monitored patients was not analyzed from the statistical point of view.

In this study it was established that most frequently a short-term ROSC was achieved (n==186; 84, 2%), but the number of patients who were discharged from the hospitals after performing of CPR was small (n=35; 15, 8%).

DISCUSSION

Cardiopulmonary resuscitation as one of the most important emergency action in life – threatening conditions is known for more than 50 years (5), but the number of survived patients does not increase significantly. There are very few studies related to the efficiency of in-hospital CPR. The aim of this study was to assess the results and their determining factors of the in-hospital CPR, such as the efficiency of CPR depending on the monitoring possibilities, various pathogenetic mechanisms of the cardiac arrest and the time of the day, when cardiac arrest has occured. The demographic characteristics of patients corresponded with the general demographic trends. There were 48% female and 52% male. The largest group of patients was between the ages of 50 to 89 years and, although the average age between the genders did not differ much (it was 68 years for females, 65 years for males), there were more females in the age group above 79 years. The most common cause of cardiac arrest was on cardiac origin, which also corresponds with the statistic data of the Republic of Latvia (19).

It was established that the majority of patients (n=214) at the time of cardiac arrest and cardiopulmonary resuscitation were located in intensive care setting, but in general ward setting the CPR was performed only on seven (n=7) patients, a short-time ROSC was achieved in 5 cases. From the total of 221 patients a short-term ROSC was achieved for 65 patients, but 35 patients (15,8%) were discharged from the hospital. Unfortunately there was no accurate information on the neurological outcome in medical records at the time of discharge. These figures can be compared with the data published in separate studies. The report from the National Registry of Cardiopulmonary resuscitation shows, overall, 44% of adult in-hospital cardiac arrest victims had a return of spontaneous circulation (ROSC); 17% survived to hospital discharge (13).

Analyzing the cardiopulmonary resuscitations performed in the general ICU and the cardiological ICU, the obtained data showed better results on ROSC in the cardiological wards. This fact requires the further analysis. However, the most frequent heart rhythm in the cases of a sudden cardiac arrest is the ventricular fibrillation or pulseless ventricular tachycardia, which reacts well to defibrillation if diagnosed in time.

The high number of cases in the general ICU – 72% (n=111), when the blood circulation did not restore, presumably was related to multimorbid patients and the long term patients and requires a further analysis.

Analyzing the ROSC frequency depending on the pathogenetic mechanism of the cardiac arrest it is evident that the best results can be achieved if the first rhythm was the ventricular fibrillation or pulseless ventricular tachycardia, which corresponds with the data available in the literature. Report from Meaney PA et al. shows that survival to hospital discharge was substantially more likely when the first documented rhythm was shockable rather than nonshockable, and slightly more likely after PEA than asystole. Survival to hospital discharge was less likely following PEA/asystole with subsequent VT/VF compared to PEA/asystole without subsequent VT/VF. First documented pulseless rhythm was ventricular tachycardia in 7%, ventricular fibrillation in 17%, pulseless electrical activity in 37% asystole 39%. Survival to hospital discharge rate was not different between those with first documented VF and VT (37% each, adjusted odds ratio [OR]) 1.08; 95% confidence interval [CI] 0.95–1.23). Survival to hospital discharge was slightly more likely after PEA than asystole (12% vs. 11%, adjusted OR 1.1; 95% CI 1.00–1.18). Survival to discharge was substantially more likely after first documented VT/VF than PEA/asystole (adjusted OR 1.68; 95% CI 1.55–1.82). Survival to discharge was also more likely after PEA/asystole without subsequent VT/VF compared with PEA/asystole with subsequent VT/VF (14% vs. 7% for PEA without vs. with subsequent VT/VF; 12% vs. 8% for asystole without vs. with subsequent VT/VF; adjusted OR 1.60; 95% CI, 1.44–1.80). But the most frequently diagnosed rhythm – the pulseless electrical activity or asystole, might indicate that the cardiac arrest was diagnosed late (8).

Analyzing the efficiency of cardiopulmonary reanimation depending on the time of day it was established that there is no statistically valid difference in the results, namely, it is not affected by the decreased number of personnel during the evening and night hours, as well as the possible exhaustion of the personnel and other factors. Abella BS et al. report (1) shows that 46, 3 % of cardiac episodes occurred in the daylight from 06:00-18:00, but 53, 7 % - in the evening and night hours. Peberdy`s et al. report (14) shows, that among in-hospital cardiac arrests occurring during day/evening hours, survival was higher on weekdays

(20.6 %) than on weekends (17.4%); whereas among in-hospital cardiac arrests occurring during night hours, survival to discharge was similar on weekdays (14.6%) and on weekends (14.8%). Our study shows that more episodes of cardiac arrest were registered in evening, but most frequently ROSC was achieved in the daylight.

The following problems were established during the study: 1) improvements are necessary in documentation of resuscitation efforts. It was difficult to collect a correct information about CPR performed on the non-monitored patients in the general profile wards; 2) there were no possibilities to analyze the records of monitors during the CPR because they were not added or recorded in the medical documentation; 3) the research was difficult to perform due to the descriptions of the cardiopulmonary resuscitations in the free style, which were frequently short of important information needed for the analysis of the CPR process.

CONCLUSIONS

There is a statistically valid difference among the CPR performed in the intensive therapy wards of various profiles. Most frequently the blood circulation is restored if the first rhythm established is the ventricular fibrillation/ pulseless ventricular tachycardia, but the most frequent rhythm is the pulseless electrical activity and asystole. The cardiac arrest time has no influence on the efficiency of cardiopulmonary resuscitation.

Conflict of interest: None

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Table 1. ROSC frequency in the ICU

	ROSC achieved		ROSC not achieved	
	n	%	n	%
General ICU	16	27	111	72
Emergency departament	9	15	32	21
Cardiological ICU	35	58	11	7
TOTAL (monitored)	60	28	154	72

n-number of patients

Table 2. ROSC episodes depending on the cardiac arrest mechanism

	ROSC achieved		ROSC not achieved	
	n	%	n	%
VF/VT	43	55,8	15	8,6
BEA/ asystole	30	39	156	89,1
Indeterminate Rhythm	4	5,2	4	2,3
Total of Episodes	77	100	175	100

n – number of CPR episodes

Table 3. ROSC depending on the time of day

	ROSC achieved		ROSC not achieved	
	n	%	n	%
06.00 - 18.00	44	66.7	99	53.2
18.00 - 06.00	22	33.3	87	46.8
Total	66	100	186	100

n – number of CPR episodes

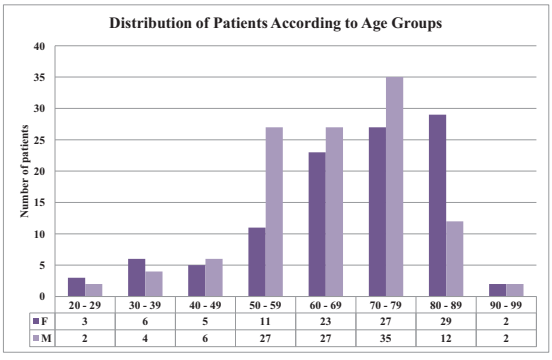

Fig. 1. Distribution of Patients According to Age Groups

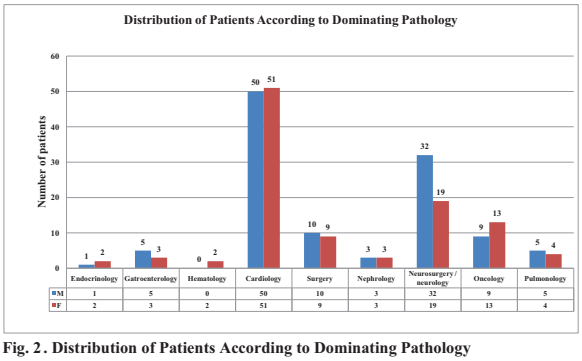

Fig. 2. Distribution of Patients According to Dominating Pathology

ORIGINAL ARTICLE

Multiple Organ Distress Syndrome Severity in Hyperglycemic and Normoglycemic Critically Ill Patients

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Summary
Introduction. Hyperglycemia, prevalent in surgical and therapeutical critical care patients, even without previous history of diabetes, requires immediate and adequate response due to its serious influence on clinical outcomes.
Aim of te study. To explore blood glucose (BG) level and glucose variability (GV) association with multiple organ distress syndrome (MODS) severity, duration, length of stay and hospital mortality in intensive care unit (ICU) patients with conventional insulin therapy.
Materials and methods. A retrospective analysis of 28 critically ill patients was carried out in a local mixed ICU. According to Surviving Sepsis campaign BG control recommendations, patients were divided into cohorts- hyperglycemic (BG >8,3 mmol/l) and normoglycemic (≤8,3 mmol/l) group. MODS severity between groups was evaluated by comparing: Sequential Organ Failure Assessment (SOFA) scores daily for 7 days; the number of patients with SOFA ≤5 each day; organ failure free days (the number of days with each SOFA criterion ≤2 and total SOFA ≤5). GV association with MODS was evaluated by performing a correlation analysis between BG measurements' standard deviation and total SOFA score. Hospital mortality was calculated in each cohort.
Results. SOFA score values in the first 5 days were practically equal in both groups, but on the 6th and 7th day it was higher in hyperglycemic than in normoglycemic group (median [interquartile range]) -6th day – 7 [4,0-7,0] vs. 3 [1,5-3,5], p=0,016 and 7th day- 4 [3,0-6,0] vs. 3 [2,0-5,0], p=0,009. The number of organ failure free days was lower in hyperglycemic group- 21/97 (22%) vs. 33/74 (46%), p=0,002. Mortality was higher in hyperglycemic group- 10/15 (67%) vs. 3/13 (23%), p=0,029. GV positively correlated with total SOFA score- rs=0,44, p=0,020 in whole population and rs=0,62, p=0,057 in hyperglycemic non-diabetic patients.
Conclusions. Hyperglycemia is higher in severely ill patients. MODS in normoglycemic patients resolves faster than in hyperglycemic. Hospital mortality is appreciably higher in hyperglycemic patients. BG variability positively correlates with MODS severity. High BG concentration and variability positively correlate with MODS severity in patients with no previous history of diabetes mellitus.
Key words: stress hyperglycemia; SOFA; MODS; mortality.

INTRODUCTION

Hyperglycemia is prevalent in surgical and therapeutical critical care patients, even without previous history of diabetes (5).
Stress-induced epinephrine, cortisol and glucagon increased secretion stimulates gluconeogenesis and tissue insulin resistance that lead to hyperglycemia (13,10).
High blood glucose concentration had been proved to cause endothelium dysfunction by increasing oxidative stress, which is one of the factors that induce organ injuries (9,12). Hyperglycemia is associated with increased surgical site infection risk (8), increased risk of death after myocardial infarction (2) and cerebrovascular events (6). Furthermore it was proved that glucose variability (measurements' irregularity), independently of glucose levels, is associated with hospital mortality in septic patients (1), but high glucose variability combined with high mean glucose values is associated with highest ICU and hospital mortality (7).
Until nowadays it is still unclear "which came first, the chicken or the egg?"- whether it is critical illness that causes hyperglycemia, or vice-versa high blood glucose leads to multiple organ failure. In our study we tried to get a little bit closer to the answer to that eternal question.

AIM OF THE STUDY

The objective of this study was to explore blood glucose level and glucose variability association with multiple organ distress syndrome severity, duration, length of stay and hospital mortality in ICU patients with conventional insulin therapy.

MATERIALS AND METHODS

A retrospective cohort study was carried out in Pauls Stradins Clinical University Hospital general intensive care unit in December 2010- January 2011.
Riga Stradins University Ethics Committee granted ethics approval for carrying out the study.
The entry criterion for the protocol was at least 2 blood glucose measurements during the first ICU hospitalization day. Patients, who were admitted to the ICU for less than 24 hours, were excluded from the study. Data were obtained from medical charts by completing the protocol maximum for 7 admission days or until patients' discharge or death.
Blood glucose measurements were obtained from venous blood samples and analyzed by hospital laboratory. In some cases point-of-care glucose meters were used.
According to Surviving Sepsis campaign BG control recommendations (4), patients were divided into cohorts: hyperglycemic (BG median >8,3 mmol/l)

and normoglycemic (BG median ≤8,3 mmol/l) group. Glucose medians were calculated in a whole admission period.

MODS severity was evaluated by using Sequential Organ Failure Assessment (SOFA) score (14). The total daily SOFA was calculated by using 24-hour most abnormal parameters. The Glasgow Coma score evaluating central nervous system function was not calculated due to its unreliability, as it is mentioned in other studies (3).

MODS severity was evaluated by comparing total SOFA medians; mean daily SOFA scores in all the patients together; the number of patients with SOFA ≤5 each day; organ failure free days (the number of days with each SOFA criterion ≤2 and total SOFA ≤5). These values were chosen and adapted from Chase J.G. *et al.* study (3).

Glucose variability was evaluated by calculating each day all BG measurements' standard deviation (SD). BG variability association with MODS was evaluated by performing a correlation analysis between SD and total SOFA score. To find out whether it depends on any specific diagnose (such as diabetes), correlation coefficient was also calculated for each diagnose separately.

Hospital mortality was evaluated by calculating mortality in each cohort and by performing Kaplan-Meier analysis and Cox's F test.

All data were checked for normality with Kolmogorov-Smirnov test and expressed as 'median [interquartile range]' where appropriate.

Variables were compared using Mann-Whitney U test, Wilcoxon Matched pairs test, Wald-Wolfowitz runs test, Fisher exact test where appropriate.

Correlation analysis was performed by using Spearman rank test.

Microsoft® Excel® 2010 and StatSoft® STATISTICA® 8.0 (2007) were used for data processing and statistical comparisons. P values < 0,050 were considered significant.

RESULTS

28 critically ill patients were included in the study. Clinical details and comparison of 15 hyperglycemic patients against 13 normoglycemic are shown in Table 1. Considerably higher median age was observed in hyperglycemic cohort- 72 [65,0 - 80,0] vs. 59 [51,0 - 68,0] years old, p=0,020. Partial oxygen pressure was appreciably higher in normoglycemic group- 114 [95,0 - 125,0] vs. 95 [79,0 - 106,0] mmHg in hyperglycemic, p=0,050. Difference in arterial blood oxygen saturation measurements between both cohorts is statistically, but not clinically significant. No statistically significant differences are seen in median SOFA scores, nevertheless, tendencies of higher SOFA are present in hyperglycemic group. Mean daily SOFA scores were analyzed and results are shown in Table 3. The difference between groups can be seen starting from the 6th day, when total SOFA score is much lower in normoglycemic cohort. The number of organ failure free days is more than 2 times higher in normoglycemic group- 33/74 (46%) vs. 21/97 (22%) in hyperglycemic, p=0,002.

General diagnoses can be seen in Table 2. There is almost no difference between groups, except diabetes mellitus, where all the patients are hyperglycemic.

Figure 1 shows differences in number of patients with total SOFA score ≤5 each day. These data are fitted with logarithmic curves for clarity. It is seen that number of patients with low SOFA grows considerably faster starting from the 4th day in normoglycemic group, but not in hyperglycemic.

Table 4 presents outcome data comparison. No differences in length-of-stay were seen, but hospital mortality was almost 3 times higher in hyperglycemic cohort- 10/15 (67%) vs. 3/13 (23%) in normoglycemic. Kaplan-Meier survival analysis had been performed and results are seen in Figure 2- much lower cumulative proportion surviving in hyperglycemic group.

Table 5 shows correlation analysis results. Glucose variability positively correlates with MODS severity in overall population- r_s=0,44, p=0,020, but no statistically significant result is seen is each cohort.

As almost all the diagnose groups in both cohorts are less than 5 patients each, it was decided to combine all non-diabetic patients in one group, to compare whether 'Diabetes' diagnose influence the results. It was found out, that glucose variability does not correlate with multiple organ distress syndrome severity in diabetic patients, and total population results are got at the cost of non-diabetic patients only, where correlation in hyperglycemic group is- r_s=0,62, p=0,057.

DISCUSSION

The results of the present study show that blood glucose concentrations are higher in patients with more severe multiple organ failure that goes with results of other studies and proves the fact that multiorgan failure is associated with widespread microvascular endothelial dysfunction caused by hyperglycemia-associated oxidative stress (5). It was found that hyperglycemia resolves faster and for more patients in normoglycemic cohort- after 6th ICU admission day median SOFA score is much lower in patients with normal blood glucose measurements and after 4th day the number of patients with low total SOFA score is higher than 70%. P value is not statistically significant due to first three days, when the amount of patients without multiple organ failure is lower in normoglycemic cohort. Amount of organ failure free days is also two times higher in normoglycemic patients.

The difference in length-of-stay was not found and it can be explained by fact that patients' physiologic condition was observed not for all hospital admission period but for 7 days only and nothing about their further condition is known.

The results of this study indicate that hospital mortality is almost three times higher in hyperglycemic group that agrees with and once more proves the results of other studies that hyperglycemia is an independent indicator of overall risk of death (5).

This study has shown that blood glucose variability positively correlates with multiple organ distress syndrome severity in whole examined population,

although no differences between hyperglycemic and normoglycemic cohorts were found. Furthermore it was found that glucose standard deviation correlates with SOFA score in patients with no previous history of diabetes only, particularly in hyperglycemic ones. Above-mentioned confirms the results of other studies that chronic hyperglycemia sets up a pattern of cellular conditioning that might be protective of acute hyperglycemia-mediated damage during critical illness, as a result, hyperglycemia and high glucose variability is more harmful in non-diabetic patients (5).

Several studies show that hypoglycemia is an independent indicator of overall risk of death (5) and that is why can influence the results of mortality rate, but no episodes of hypoglycemia were observed during our study.

It is clear that nutrition type and introduced calories' amount influence blood glucose significantly, but these factors were found to be similar in all patients and that is why were not taken into account during our study.

Corticosteroid therapy usage was not registered in the protocol and not analyzed, because newest studies show that exogenous steroids have low impact on suppression of insulin sensitivity in critically ill patients due to already increased levels of catecholamines and cortisol (11).

Our findings have several important clinical implications. First, they approve once again that high blood glucose levels should be adequately managed in critically ill patients. Second, that glucose acute fluctuation must be avoided e.g. – special insulin introduction protocols should be used. Third, that high blood glucose levels and variability are more dangerous in patients with no previous history of diabetes and that is why should be managed with special attention.

Despite everything, we still don't know the main cause of stress hyperglycemia's vicious circle, and that is why further research is required.

CONCLUSIONS

This paper presents results from retrospective cohort study that evaluates blood glucose and glucose variability association with multiple organ distress syndrome severity, duration, length of stay and hospital mortality in critically ill patients with conventional insulin therapy. Several main conclusions are drawn from this analysis.

First, this study demonstrates that hyperglycemia is higher in severely ill patients.

Second, multiple organ distress syndrome severity in normoglycemic critically ill patients resolves faster than in hyperglycemic.

Third, it was shown that hospital mortality is appreciably higher in hyperglycemic patients.

Forth, blood glucose variability positively correlates with multiple organ distress syndrome severity.

Fifth, high blood glucose concentration and variability positively correlate with MODS severity in critically ill patients with no previous history of diabetes mellitus.

Conflict of interest: None

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Table 1. Comparison of protocol variables

Total patients	28	15	13	
Number of males	17 (59%)	8 (53%)	9 (69%)	0,460 [a]
Age, years	66 [53,5 - 73,5]	72 [65,0 - 80,0]	59 [51,0 - 68,0]	0,020
$P_{systolic}$, mmHg	130 [126,5 - 152,3]	129 [124,5 - 156,5]	133 [128,0 - 151,0]	0,434
$P_{diastolic}$, mmHg	64 [59,3 - 68,0]	62 [59,0 - 65,0]	68 [62,0 - 72,0]	0,065
MAP, mmHg	88 [81,2 - 95,5]	86 [78,3 - 93,3]	89 [85,0 - 98,3]	0,140
P_aO_2, mmHg	104 [88,5 - 118,4]	95 [79,0 - 106,0]	114 [95,0 - 125,0]	0,050
PCO_2, mmHg	41 [34,6 - 45,5]	37 [33,6 - 43,7]	42 [39,8 - 47,3]	0,076
F_IO_2	0 [0,4 - 0,6]	1 [0,4 - 0,7]	0 [0,4 - 0,5]	0,107
S_aO_2, %	98 [97,2 - 98,1]	98 [96,4 - 98,0]	98 [97,6 - 98,3]	0,053
Hemoglobin, g/L	107 [95,5 - 118,5]	107 [97,0 - 111,0]	112 [94,0 - 120,0]	0,596
Blood glucose, mmol/l	8 [7,6 - 11,3]	11 [8,7 - 12,6]	8 [7,2 - 7,9]	0,000
Glucose SD, mmol/l	1,4 [1,0 - 2,7]	2,4 [1,4 - 3,4]	1,0 [0,77 - 1,43]	0,001
Insulin, U/h	0 [0,0 - 2,0]	2 [0,4 - 3,5]	0 [0,0 - 0,0]	0,002
P_aO_2/F_IO_2, mmHg	232 [167,8 - 323,6]	185 [143,6 - 282,8]	271 [227,5 - 327,1]	0,062
Platelets, 10^3/ mm^3	166 [133,5 - 229,3]	154 [110,0 - 222,5]	188 [135,0 - 322,0]	0,504
Total bilirubin, µmol/l	12 [10,0 - 20,5]	14 [10,0 - 20,5]	11 [9,0 - 16,5]	0,443
Creatinine, µmol/l	88 [66,0 - 110,0]	94 [81,5 - 109,0]	79 [49,0 - 111,0]	0,289
SOFA respiratory failure	2 [1,0 - 3,0]	3 [2,0 - 3,0]	2 [1,0 - 2,0]	0,080
SOFA coagulation failure	0 [0,0 - 1,0]	0 [0,0 - 1,0]	0 [0,0 - 1,0]	0,908
SOFA hemodynamic failure	3 [0,0 - 4,0]	3 [0,0 - 4,0]	2 [0,0 - 4,0]	0,519
SOFA hepatic failure	0 [0,0 - 0,0]	0 [0,0 - 0,0]	0 [0,0 - 0,0]	0,836
SOFA renal failure	0 [0,0 - 0,5]	0 [0,0 - 0,0]	0 [0,0 - 1,0]	0,612
SOFA	5 [4,0 - 7,3]	6 [4,0 - 8,0]	5 [3,0 - 5,0]	0,167

* P values computed between hyperglycemic and normoglycemic group using Mann-Whitney U test, or Fisher exact two-tailed test where indicated [(a)]

Table 2. Patients' distribution by general diagnosis

Total patients	28	15	13	
Pancreatitis	3 (10,3%)	1 (6,7%)	2 (15,4%)	0,583
Neurological	10 (34,5%)	4 (26,7%)	6 (46,2%)	0,433
Diabetes mellitus	5 (17,2%)	5 (33,3%)	0 (0,0%)	0,044
Respiratory	6 (20,7%)	3 (20,0%)	3 (23,1%)	1,000
Renal	2 (6,9%)	0 (0,0%)	2 (15,4%)	0,206
Other	2 (6,9%)	2 (13,3%)	0 (0,0%)	0,484

* P values computed between hyperglycemic and normoglycemic group using Fisher exact two-tailed test

Table 3. Summary comparison of MODS severity

	Hyperglycemic group	Normoglycemic group	P value *
Median SOFA score	6 [4,0 - 8,0]	5 [3,0 - 5,0]	0,167 [a]
Mean SOFA score each day:			
SOFA, day 1	6,1	5,4	0,786 [b]
SOFA, day 2	5,8	5,1	0,399 [b]
SOFA, day 3	6,0	5,5	0,289 [b]
SOFA, day 4	5,9	4,4	0,560 [b]
SOFA, day 5	5,0	4,0	0,199 [b]
SOFA, day 6	6,0	2,7	0,016 [b]
SOFA, day 7	4,4	3,8	0,009 [b]
Number of organ failure free days (%)	21/97 (22%)	33/74 (46%)	0,002 [c]

* P values computed using: Mann-Whitney U test [(a)], Wald-Wolfowitz runs test [(b)], Fisher exact two-tailed test [(c)]

Table 4. Outcome comparison

	Hyperglycemic group	Normoglycemic group	P value *
ICU length-of-stay, days	19 [8,0 - 41,0]	24 [13,0 - 43,0]	0,475 [a]
Hospital length-of-stay, days	20 [16,0 - 42,0]	32 [13,0 - 43,0]	0,747 [a]
Hospital mortality (%)	10/15 (67%)	3/13 (23%)	0,029 [b]

* P values computed using: Mann-Whitney U test [(a)], Fisher exact two-tailed test [(b)]

Table 5. Correlation analysis

	Total patients	Correlation between glucose variance & SOFA*
Overall population	28	0,44, p=0,020
Hyperglycemic group	15	0,45, p=0,091
Normoglycemic group	13	0,18, p=0,558
Diabetes mellitus patients	5	0,00, p=1,000
Non-diabetic patients	23	0,43, p=0,039
Hyperglycemic group	10	0,62, p=0,057
Normoglycemic group	13	0,18, p=0,558

* Correlation analysis performed using Spearman rank test.

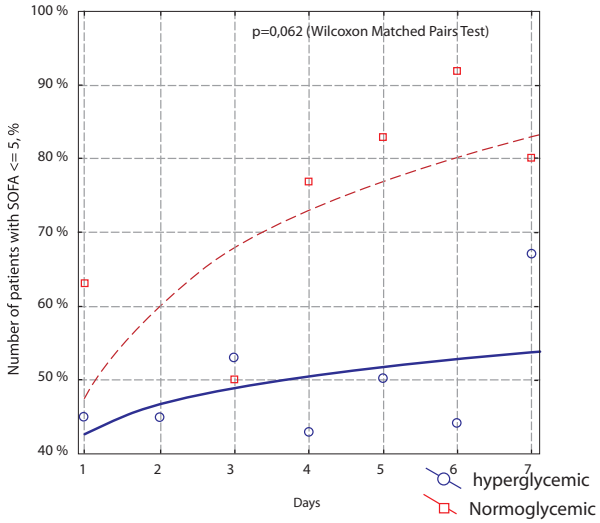

Fig. 1. Number of patients with total SOFA score ≤5 each day

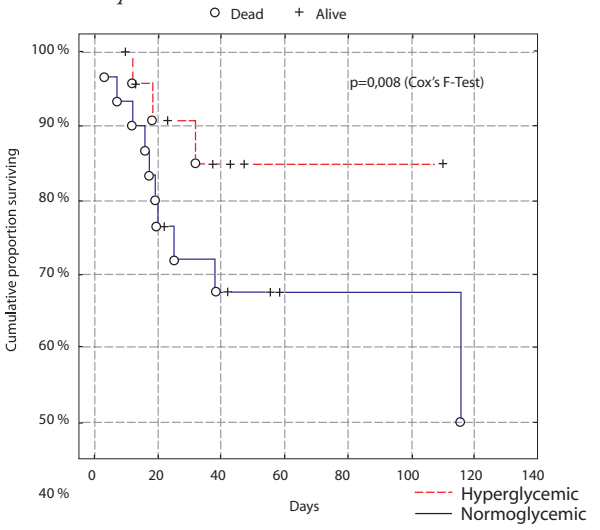

Fig. 2. Cumulative Proportion Surviving (Kaplan-Meier analysis)

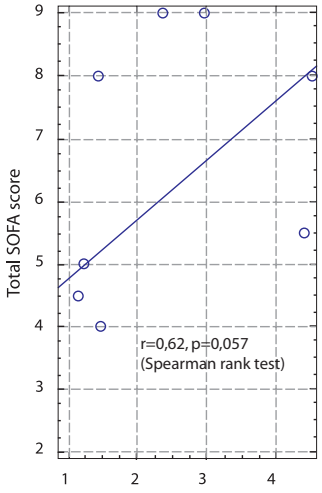

Fig. 3. Correlation analysis between glucose variability and total SOFA score in hyperglycemic patients with no previous history of diabetes

ORIGINAL ARTICLE

Aortic Valve Replacement in Latvia Influenced by an Ageing Population and Transcatheter Procedures – an Update

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Summary
Introduction. Aortic valve disease in the ageing population of the developed countries is the dominant heart valve disease affecting predominantly the elderly patients. Surgeons routinely more and more often encounter very frail, elderly patients with severe co-morbidities referred to surgery.
Aim of the study. Aim of this study is to summarize data on usage frequency of mechanical valve prostheses and bioprostheses in all aortic valve replacement operations performed during the years 2008-2010 in the Cardiac Surgery Centre of Pauls Stradins Clinical University hospital, compare it with previous data from years 2002-2006 and to evaluate the possible influence of introduction of transcatheter aortic valve implantation (TAVI) in Latvia.
Materials and methods. Data was collected from the National Database of Cardiac surgery as well as from surgery registration journals of departments in a retrospective manner. All primary data were analyzed using MS Excel software.
Results. The mean age of aortic valve replacement patients has gradually increased over years from 65.84 in 2008 to 67.35 in 2010. There has been a marked increase in the population of patients older than 80 years from 7.1% in 2008 to 10.8% in 2010 (TAVI patients not included). From 1044 aortic valve operations in 534 bioprostheses were used and in 510 cases mechanical valves were utilized. From all patients undergoing aortic valve replacement in 2008 42.3% received a bioprosthesis, this number increased to 49.4% in year 2009 and even to 60.5% in 2010.
Conclusions. Patients undergoing aortic valve replacement are getting older with each year, especially in the last few years after introduction of TAVI. There is a tremendous increase of bioprostheses usage in valve replacement operations – a consequence of an ageing population and improved durability of bioprostheses.
Key words: aortic valve replacement, aortic bioprostheses, TAVI, elderly patients.

INTRODUCTION

The changes in the adult population of developed countries with a marked shift to more elderly people have affected every area of medical and surgical practice, including cardiac surgery. More and more people are expecting to enjoy life in good quality not just into their 70s but also into their 80s and beyond. Accordingly their expectations of treatment for heart and other diseases have also shifted upwards at the same time. The field of valvular heart disease has followed this trend and has experienced important changes in the understanding of its pathophysiology, etiology and treatment. As mentioned earlier (10) there is a decline in cases of rheumatic fever in developed countries in recent times, despite that, the ageing of general population has led to no decline in the prevalence of aortic valve stenosis. Thus aortic stenosis remains a significant medical entity because of calcific aortic stenosis as the dominant aortic valve pathology. Following the raising expectations of patients, the techniques, methods and expertise in cardiac surgery have also made huge improvements to meet these expectations. Doctors routinely more and more often are asked to perform aortic valve surgery in frailer, more elderly patients with severe co-morbidities. For severe calcific aortic stenosis valve replacement is the treatment of choice. For valve replacement there are two options: mechanical heart valve prostheses and bioprostheses, each with pluses and minuses of its own, discussed elsewhere (10). The most important and serious drawback for bioprostheses is limited longevity (5, 16), as claimed in earlier studies, within 10 years of implantation 50% to even 60% of all bioprosthetic valve recipients will undergo a repeated surgical intervention caused by valve failure (8). On the contrary mechanical heart valve prostheses can last for a lifetime, but their recipients are obliged to use anticoagulants for the rest of their life complicated with a high bleeding rate. From a clinical point of view, one has to balance the severity and clinical implications of complications. The current guidelines position the mechanical valve prostheses as advantageous for aortic valve replacement in those aged <65 years and the bioprosthesis for those aged >65 years, especially if such patients do not need anticoagulation for other risk factors (1, 13). Nevertheless there has been an ever growing trend towards use of bioprostheses in younger patients, which is fueled by several factors. Recent publications suggest that second generation bioprostheses can last for twenty or even more years – including follow up data on Medtronic Hancock, St.

Jude Biocor (predecessor of St. Jude Epic), Carpentier-Edwards Standard and others (4, 6, 14). A recent study involving Hancock II prosthesis has shown freedom from reoperation at 20 years to be 86.8% in patients >60 years and 52.2% in patients <60 years of age (14). There are several studies aiming to evaluate which prosthesis type is more beneficial the latest of which is that of van Geldorp et al. (15) in which they weigh lifetime anticoagulant-related event risk against reoperation risk and conclude that even for patients aged 60 years, event-free life expectancy is better with a bioprostheses. And last but not least is the widespread clinical introduction of transcatheter aortic valve procedures. Which currently is aimed as a procedure for the ever-growing very elderly part of aortic stenosis population (12), but with time is also expected to be a safe alternative for a reoperation for younger bioprosthesis recipients thus hypothetically lowering the mortality/morbidity risk at a repeat procedure.

AIM OF THE STUDY

Aim of this study is to summarize data on all aortic valve replacement operations performed during the years 2008-2010 in the Cardiac Surgery Centre of Pauls Stradins Clinical University hospital. To compare the usage frequency of mechanical valve prostheses and bioprostheses with data from years 2002-2006, published before (10). To evaluate the influence of the introduction of TAVI in Pauls Stradins University hospital in late 2009 on prosthesis choice.

MATERIALS AND METHODS

Data was collected from the National Database of Cardiac surgery as well as from surgery registration journals of departments in a retrospective manner. All calculations to obtain relative numbers, percentages and mean values as well as their visual representations were done using MS Excel software and its integrated data analysis tool pack.

RESULTS

Altogether data over 1044 aortic valve operations has been collected. The patient mean age has gradually increased from 65.84 years in 2008 to 67.35 in 2010. From all the patients undergoing surgery in year 2008 63.7% or 233 patient were older than 65 years, but in 2010 this number increased to 246 or 69.9% consistent with the trend of an aging population. More interestingly, there has been a relatively even bigger increase in the population of patients >80 years from 7.1% in 2008 to 10.8% in 2010 (TAVI patients are not included). As can be seen in fig. 1, calcific degeneration is the leading cause for aortic replacement, encountered in more than ¾ of all the cases. As mentioned before, percentage of patients with rheumatic heart disease remains very low – less than 5 % of all patients (see fig. 1).

From all 1044 aortic valve operations in 534 bioprostheses were used and in 510 cases mechanical valves were utilized. From all patients undergoing aortic valve replacement in 2008 42.3% received a bioprosthesis, this number increased to 49.4% in year 2009 and even to 60.5% in 2010. In fig. 2 the choice of prosthesis type for aortic valve replacement in relation to the age of the patient is illustrated. This figure represents summarized data from years 2008 and 2009 (fig. 2. B) and separately for 2010 (fig. 2. C) to depict the differences after introduction of TAVI in Latvia.

Since September 2009 till 2010 TAVI has been performed in 34 high surgical risk patients with mean age >80 years and logistic EuroSCORE > 20% (7), from which 30 patients were treated in 2010. From these patients 25 were >80 years old.

DISCUSSION

Patients undergoing aortic valve surgery are getting older with each year and this can be explained by an ageing population and in the last few years also by the appearance of TAVI in clinical praxis. By the introduction of TAVI a new group of patients appears at the surgical clinic – patients who previously were never referred to surgery at all because they have been coined unsuitable for operative therapy already by their cardiologists or general practitioners due to their poor overall condition and co-morbidities. With the initial published results from large TAVI trials and registries being very good (3, 9, 11), one can expect this procedure to become even more widespread and accepted also for a younger patient population.

Usage of bioprostheses has experienced a very fast growth, from 4% in 2002 to 25% in 2006 (10) and continuously to 42.3% in 2008 and up to 60.5% in 2010 of all patients undergoing aortic valve replacement. This phenomenon may have several explanations:

- availability of new data on durability of second generation bioprostheses;
- several studies claiming a beneficial outcome after bioprosthetic aortic valve replacement already after 60 years compared to mechanical prostheses (g, un hanck);
- large trials demonstrating very good early results of TAVI and promising reports on TAVI in patients with dysfunctional bioprosthetic heart valves – the so called “valve in valve” procedures (2).

If looked more closely at the group of patients >65 years, to which the current guidelines (1, 13) recommend usage of bioprosthesis, an increadible increase has happened from 61.2% of patients receiving a bioprosthesis in 2008 to 78.6% in 2010. With this data we are nearing to that of USA with their market shares 25:75 in favour of bioprostheses (17). If looked more carefully at fig. 2 one can notice that especially in 2010 there is an increase in bioprostheses use in younger patients, which in part might be explained by introduction of TAVI as a potential alternative to future re-do surgery.

In figure 1 it can be clearly seen, that rheumatic disease counts for a very small fraction of aortic valve pathology - typical for westernized countries and following the trend seen from 2002 to 2006 (10), with calcific degeneration being the most often encounterd pathology of the aortic valve today.

CONCLUSIONS

Patients undergoing aortic valve replacement are getting older with each year, especially in the last few years after introduction of TAVI, which brought a new – much older, sicker and frailer patient population to the surgical clinic as ever before. There is a marked increase of bioprostheses usage in valve replacement operations – a consequence of an ageing population and improved durability of bioprostheses. Also much younger patients receive a bioprosthesis, which might be due to a smaller risk of reoperation because of long lasting biological prostheses and due to introduction of TAVI as a possible alternative to a reoperation.

ACKNOWLEDGEMENT

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Conflict of interest: None

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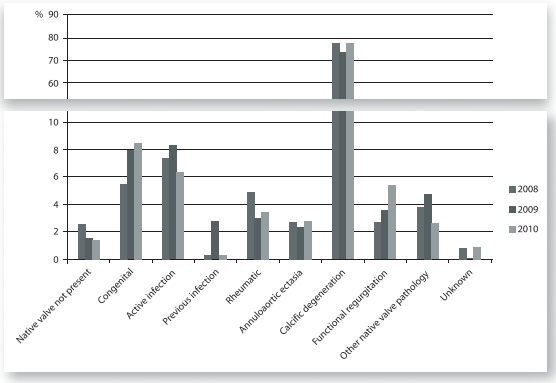

Fig. 1. Underlying pathology in patients undergoing aortic valve replacement in the Cardiac Surgery centre in percents of total operations per year

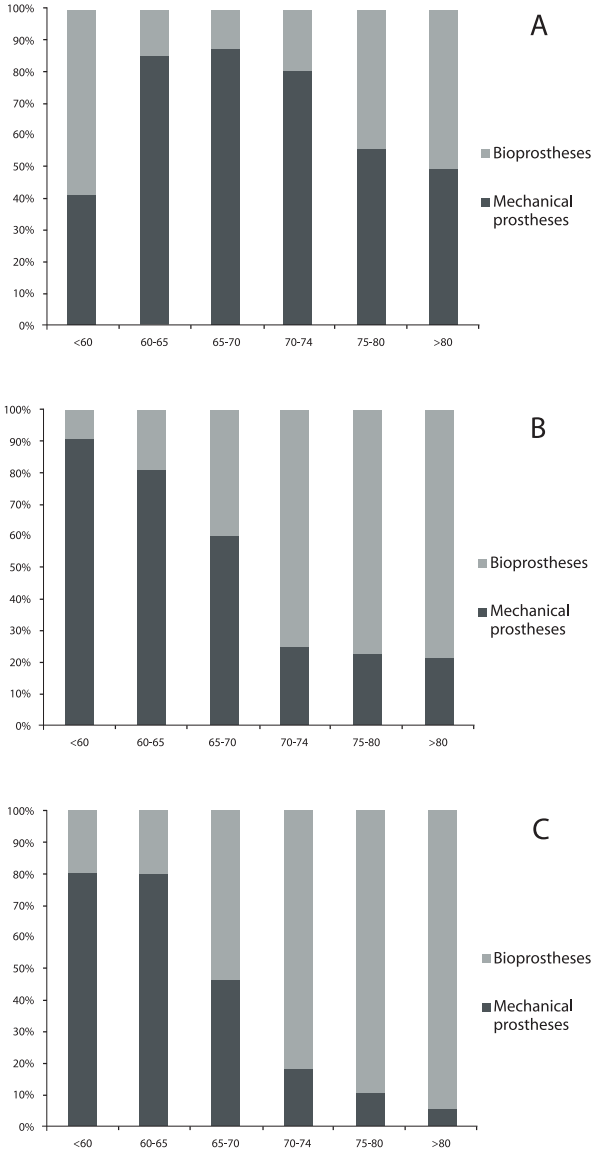

Fig. 2. Choice of a bioprosthesis or a mechanical prosthesis for aortic valve replacement in various age groups: A – years 2002-2006 (adapted from (1)), B – years 2008-2009, C – year 2010", bet būtu jābūt "Fig.2. Choice of a bioprosthesis or a mechanical prosthesis for aortic valve replacement in various age groups: A – years 2002-2006 (adapted from (10), B – years 2008-2009, C – year 2010

ORIGINAL ARTICLE

Aortic coarctation repaired within the first year of life: an 11 year review

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Summary
Introduction. Congenital heart diseases (CHD) affect 8-12 per 1000 live-born infants and it is one of the most common and serious congenital anomalies, aortic coarctation (AoCo) accounts for 6-10% of all the congenital heart diseases, and if not diagnosed early in life it can result in severe morbidity and mortality (3,9,13,11).
Aim of the study. The aim of the study was to analyze the epidemiology of AoCo in newborn in Latvia, to evaluate a single centre 11 year experience with children who underwent AoCo repair in our institution within the first year of life, to define morbidity, mortality, risk factors, short and long term results.
Materials and methods. The study was approved by the committee of ethics of the university hospital for children. Retrospective and prospective study/follow-up of all (74) patients younger than 12 months undergone native coarctation repair in our institution between January1, 2000 and December 31, 2010.
Results. The prevalence of the coarctation of the aorta in Latvia was 3.43+/-1.2 per 10 000 life born infants. Seventy-four neonates and infants were analyzed: 44 boys (59%) and 30 girls (41%). Median age at the time of primary surgical correction was 47.3+/-58 days, medium weight 4.2+/-1.6kg. Prostoglandin E1 was used in 62%, inotropic stimulation in 20% of cases, assisted ventilation in 18%. According to the anatomy of the congenital heart disease patients constituted group I- the patients with simple coarctation with and without atrial septal defect (ASD) in 57%, group II –patients with coarctation and ventricular septal defect (VSD) 23%, group III- complex coarctation 20%. The infantile juxtaductal AoCo with isthmus hypoplasia was detected in 83%(n=62), postductal AoCo in 4%(n=3) and juxtaductal membrane in 12%(n=9), hypoplasia of the aortic arch in 16%(n=12). The techniques for primary repair included the resection with simple anastomosis end-to-end (ETE) in 26% (n=19), subclvian flap aortoplasty(SFA) in 65%(n=48) and extended anastomosis end-to-end (eETE) in 8%(n=6), primary balloon angioplasty in 1 case. There were 5 cases (6,8%) of early postoperative death and 9 cases of later death. Recoarctation occurred in 14 patients (23%), all underwent balloon angioplasty with no significant residual gradient, and there was no mortality or complications after reinterventions.
Conclusions. The surgical correction of the AoCo remains gold standard for neonates and small infants. There were no statistically significant differences between the incidences of recoarctation dependent on the method of primary surgical correction. The incidence of recoarctation in cases of primary surgical correction early in life remains comparatively high. Baloon angioplasty is a method of choice in cases of recoarctation, it is safe and effective with low incidence of persistent coarctation. Mortality is most importantly influenced by preoperative status, the severity of associated anomalies, surgical outcomes, perioperative intensive treatment.
Key words: aortic coarctation, congenital heart disease.

INTRODUCTION

Congenital heart diseases (CHD) affect 8-12 per 1000 live-born infants and it is one of the most common and serious congenital anomalies. Approximately one quarter of these children will have critical CHD which requires surgery or catheter intervention in the first year of life (3, 6). The incidence of AoCo is approximately 36 (29-49)/100 000 infants. AoCo accounts for 6-10% of all the congenital heart diseases (3, 9, 11, and 13). According to the data from European surveillance of congenital anomalies the prevalence of AoCo excluding chromosomal anomalies during years from 2005 to 2009 in summary from all the registries ranged 2.49-3.01 per 10 000 live births (16). There is a prevalence of infantile type coarctation with variable degree of the hypoplasia of aortic arch in infants. It leads to the development of severe left heart failure after the closure of the *ductus arteriosus* with subsequent circulatory shock, acidosis, renal insufficiency and death if left untreated. Ductal dependent coarctation may require early surgical intervention. If not diagnosed early in life, it can result in severe morbidity and mortality. In approximately 64% of the infants with AoCo it manifests as a leading CHD soon after the birth (14). Due to different anatomy and possible associated anomalies, there are several methods of surgical correction of the disease. The surgical correction of the coarctation of the aorta is the standard method in neonates and infants (surgical techniques used are *anastomosis end-to-end (ETE)*, *subclavian flap angioplasty (SFA)* and *extended end*-to-*end anastomosis (eETE)*) (2,3,7,9). The results of primary balloon angioplasty are debatable in early age and more associated with the risk of aneurism formation, recoarctation and possible injury of femoral arteries and

sub sequent stenosis of femoral arteries. There is high incidence of recoarctation - up to 20-40% in cases of coarctation repaired within the first year of life with the need for reinterventions- balloon angioplasty of recoarctation site. Coarctation of the aorta is associated with increased risk of arterial hypertension in further life despite successful repair and shortened life expectancy (1, 8). According to the literature, an early correction of the coarctation preserves the vessels of the postcoarctation zone from structural changes but the precoarctation zone remains structurally changed with the thickened intima and media as well as increased amount of collagen and elastin. Complications such as recoarctation or secondary hypertension, probably related to the loss of arterial elasticity, frequently occur after aortic coarctation surgery (1, 15).

AIM OF THE STUDY

Aim of the study was to explore the epidemiology of the coarctation of the aorta in newborn infants in Latvia. To analyze all the cases of aortic coarctations diagnosed and operated in our clinics within the first year of life in the period of time from January 1,2000- December 31, 2010 to evaluate the risk factors for recoarctation, morbidity, mortality, short and long term results and the factors affecting the outcomes.

MATERIALS AND METHODS

The study was approved by the committee of ethics of the university hospital for children. The study was designed as a single-centre, clinical, retrospective and observational trial. The analysis of the medical records, echocardiographies, angiographies of all the neonates and infants in the age group up to twelve months diagnosed AoCo and undergone surgical and interventional correction of the coarctation in our institution between January 1, 2000 and December 31, 2010 was carried out. To analyze the epidemiology of AoCo in newborn infants in Latvia we analyzed and compared the data of all the neonates and infants up to the age of 12 months treated in our institution with the diagnosis of AoCo in the periods of time from January 1, 2000-December 31, 2004 and the period of time from January 1, 2005-December 31, 2010 in correlation to the birth rates in our country within these years. The patients were divided into 3 groups according to additional cardio-vascular diagnosis: group I- the patients with simple AoCo with and without ASD, group II –patients with AoCo and VSD, group III- complex coarctation (AoCo in combination with different intracardiac lesions). The follow up of the patients lasting 13-124 months was carried out (mean follow up time 66, 32+/34 months (median 59, 5, mode 52 months). For the data storing and processing the Microsoft Office Excel 2003 program was used. Statistical analysis was performed with SPSS 16. The anthropometric and clinical characteristics were summarized as means and standard deviations and as a percentage of the group for categorical variables. Pearson's chi-square test and Fishers exact tests were used to compare the groups of patients. The p value<0. 05 was considered statistically significant. Correlations were calculated by Spearman's rank correlation coefficient.

RESULTS

Our institution-the clinic for pediatric cardiology and cardiac surgery of the University Hospital for Children in Riga is the only institution in our country where congenital heart diseases in pediatric patients are treated so our data represent the overall data of the population of our country. The birth rates in our country within the years 2000-2010 were 21 197+/-1212 life born infants per year (49), the prevalence of the AoCo was 3.43+/-1.2 per 10 000 life born infants (in the period of time from 2000-2004 it was 2.56+/-0.86, but in the period of time from 2005-2010 4.1+/-1.1 per 10 000 life born infants).

74 neonates and infants were enrolled in the study group (figure 1.). The patients with hypoplastic left heart syndrome were excluded from the study group. There were 44 boys (59%) and 30 girls (41%), 59% (n=44) of all the patients were newborns. Median age at the time of primary surgical correction was 47.3+/-58 days (in the period of time from year 2000-year 2004 it was 68.7+/-67.3 days, but within years 2005-2010 37.65+/-51.6 days, p=0,033), medium weight at the time of primary correction of AoCo was 4.2+/-1.6kg. The indication for repair was conservatively untreatable heart insufficiency. Antenatal diagnosis was detected in 19% of the cases (4% within the period of time form year 2000-2004, but 25% in the period of time from 2005-2010, p=0.032). There were 45 patients at the age group up to 2 months old (61%) (the patients considered to have ductus dependent AoCo) and prostoglandin E1 was used in 62%. The babies were in need of inotropic stimulation in 20% of cases, assisted ventilation 18% and there were no statistically significant differences between the need of intensive care within the study period (p>0.05). During the period of time from 2005-2010 64%(n=29) of the patients were sent by maternity hospitals, but 36%(n=16) were referred by general practitioner or emergency department after the discharge from the maternity hospital. There were other diagnoses instead of congenital heart disease suspected in 27% in the age group up to two months; they were septicemia, pneumonia and feeding disturbances. There was a correlation between antenatal diagnosis and concomitant intracardiac pathology observed in the group of patient up to 2 months old (r=0,407, p=0.06, n=45).

According to the anatomy of the CHD patients constituted group I- the patients with simple corctation with and without ASD in 57%(n=42) (45% of newborns, but 73% of infants up to 12 months, p=0.02), group II –patients with coarctation and VSD in 23%(n=17) (34% newborns, but 7% in older infants, p=0.02), group III- complex coarctation in 20% (n=15) (equal in both groups)(table1.).The infantile juxtaductal AoCo with isthmus hypoplasia was detected in 83%(n=62), postductal AoCo in 4%(n=3) and juxtaductal membrane

in 12%(n=9), hypoplasia of the aortic arch (transverse arch below -2 z score according to patients body surface area)in 16%(n=12). Bicuspid aortic valve was detected in only 8%(n=6) patients of the study group. Elevation of blood urea levels (median 9.82+/-2.21, normal range 2.5-6.4mkmol/L) was detected in 15%(n=11) and elevated creatinine in 15%(n=11)(medium 139,95+/-20.12 mkmol/L, normal range 18-35 mkmol/L) patients in the period prior to the operation.

The techniques for primary repair included the resection with *ETE* in 26% (n=19), *SFA* in 65%(n=48) and *eETE* in 8%(n=6), primary balloon angioplasty in 1 patient. The intraoperatively resected segments of coarctation were sent for pathohistological examination and the characteristic changes were detected: intimal proliferation, fibroelastosis, disruption of elastic tissue, fibrointimal thickening (picture 3).

There were 5 cases (6,8%) of early postoperative death(within 30 days following the surgery: 1 case of septicaemia and 4 cases of cardio-vascular insufficiency (all the cases were neonates) and 9 cases of later death in the study period: 1 renal insufficiency, 1 case of pulmonary hypertension and pneumonia, 2 cases of endocardial fibroelastosis, 3 cases of sudden death (no results of autopsies available), 1 case of severe cardiovascular insufficiency and acidosis and 1 patient died after the surgical correction of combined intracardiac lesion. No paraplegia and intracranial bleeding occurred. Kaplan-Meier survival curve for these patients is shown in figure 2. In the group of patients constituting lethal cases within the study period there were more cases of antenatal diagnosis (p=0.02), more frequent use of inotropes (p=0.03) and assisted ventilation (p=0.013), more often elevated urea levels (p=0.028), hypoplastic aortic arches and concomitant intracardiac pathologies observed, but there was no correlation with the method of surgical correction observed (table 2.). There were 60 patients further followed up. Slight shortening of the left arm as the result of subclavian steal was found in 2 patients and asymmetry of palms in1 in the group of Waldhausen operation (7, 7%). During the follow-up period recoarctation (mean pressure gradient >20mmHg at rest in descending aorta in echocardiography) occurred at the age from 2 months to l8 years (2 -96 months, mean 38,21+/-32,8months) in 14 patients (23%) (the lethal cases excluded)(in 73% these patients were primary repaired as neonates). There were no statistically significant differences between the incidence of recoarctation dependent on the method of primary surgical correction (18% in ETE group, 26% in SFA group and 25% in extended anastomosis group (p>0.05)(figure 3.). All of them underwent balloon angioplasty (one of the patients twice) with no significant residual gradient; one patient (8 years old) required also implantation of 2 stents due to recoarctation and hypoplastic transverse aortic arch. There was no mortality or complications after reinterventions. The actuarial survival curves show differences between survivals in groups I, II and III (figure 4). The patients age at the end of follow-up period was 66, 32+/-34 months (1 year 1months-10years10months). There was a need of antihypertensive treatment in 8 % of the patients without hemodinamically significant residual gradient.

DISCUSSION

Our data show that the prevalence of the AoCo does not significantly differ from the data of literature although there are differences between more recent data and the data from the first half of the last decade. AoCo still carries high mortality rates between neonates and small infants. Mortality is most importantly influenced by the preoperative status, the severity of associated intracardiac anomalies and perioperative intensive treatment. In the group of patients constituting lethal cases within the study period there were more cases of antenatal diagnosis (p=0.02), more frequent use of inotropes(p=0.03) and assisted ventilation(p=0.013), more often elevated urea levels(p=0.028), hypoplastic aortic arches and concomitant intracardiac pathologies observed which is consistent with the data from literature.

Prenatal diagnosis of aortic coarctation suffers from high false-negative rates at screening and poor specifity, therefore AoCo is most common duct-dependent cardiac defect missed at routine physical screening of the newborn. During the period of time from 2005-2010 36% of the patients in the age group up to 2 months were referred by general practitioner or emergency department after the discharge from the maternity hospital and there were other diagnoses instead of congenital heart disease suspected in 27% which is indicative of the need for further education for general practitioners and pediatricians working with neonates and small infants.

The vast majority of the patients were operated by SFA (in 65%(n=48)) but eETE in only 8%(n=6)of the cases therefore statistical comparison which technique is superior cannot be made. The coarctation repaired in neonates and small infants carries high recoarctation rate (23% in our study, all the lethal cases excluded) which is comparable with the reports from literature and the recoarctation rates did not differ significantly between the methods. The data from literature confirm surgery as a method of choice for neonates and small infants in cases of AoCo. A limited number of studies comparing surgery and balloon dilatation showed significantly lower reintervention and complication rates after surgery than after balloon angioplasty in this age group. Baloon dilatation and/or stent implantation as a primary repair is mainly recommended for older children due to the need for frequent redilatation in growing children, high incidence of intimal proliferation in stents and potential aneurysm formation (16).

CONCLUSIONS

The surgical correction of the AoCo remains the gold standard for neonates and small infants. There were no statistically significant differences between the incidences of recoarctation dependent on the method of primary

surgical correction. The incidence of recoarctation in cases of primary surgical correction early in life remains comparatively high. Balloon angioplasty is a method of choice in cases of recoarctation, it is considered to be safe and effective with low incidence of persistent coarctation. Mortality is most importantly influenced by preoperative status, the severity of associated anomalies, surgical outcomes, perioperative intensive treatment. The effect of scarifying left subclavian artery in neonates and small infants is debatable. There were no severe ischemic complications of the left arm and left hand connected with subclavian flap aortoplasty retrospectively.

Conflict of interest: None

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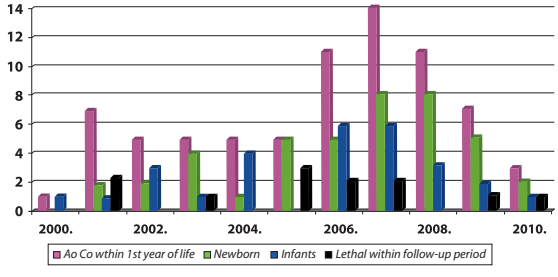


Fig. 1. Patients with AoCo corrected within the first year in life (n=74).

Table 1. The groups of patients according to concomitant intracardiac pathology.

Group of patients	Intracardiac pathology	Number of patients
Group I	None(isolated coarctation)+/- ASD	42(57%)
Group II	AoCo+VSD	17(23%)
Group III	Complex coarctation:	15(20%):
	Double inlet left ventricle+VSD	1
	Subvalvular aortic stenosis+VSD	1
	Subvalvular aortic stenosis	4
	Valvular aortic stenosis	4
	Supravalvular aortic stenosis	1
	Mitral valve insufiiciency(valve pathology)	1
	Mitral stenosis+VSD	1
	Atrio-ventricular septal defect	1
	Pulmonary stenosis+ASD	1

Table 2. Factors affecting the lethal outcome.

		Alive	Lethal outcome during study period	P value
Antenatal diagnosis	Yes	8 (13%)	6 (43%)	0.01
	No	52 (87%)	8 (57%)	
Use of inotropes	Yes	9 (15%)	6 (43%)	0.02
	No	51(85%)	8 (57%)	
Assisted ventilation	Yes	7 (12%)	6 (43%)	0.006
	No	53(88%)	8 (57%)	
Elevated blood urea levels	Yes	54(90%)	9(64%)	0.015
	No	6(10%)	5(36%)	
Hypoplastic aortic arch	Yes	4(7%)	8(57%)	0.001
	No	56(93%)	6(43%)	

*Tests of significance were chi-square test and Fisher's exact test. NS-not significant, P>0.05.

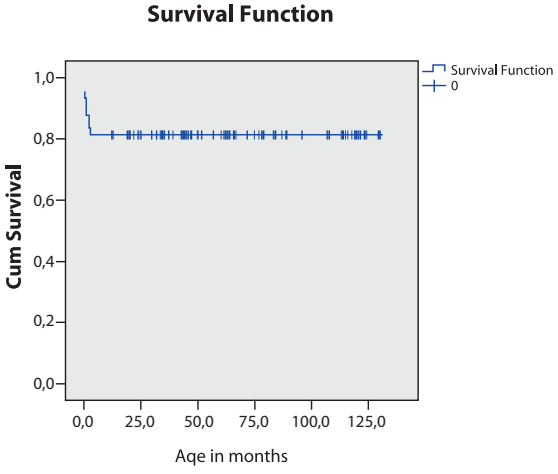


Fig. 2. Kaplan-Maier survival curve for the patients operated with AoCo within the first year of life in the priod of time from year 2000-2010.

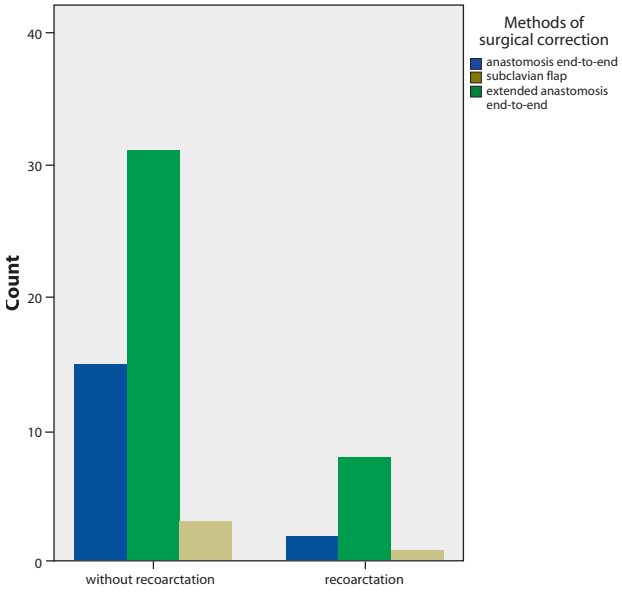


Fig. 3. Recoarctation and the method of primary surgical correction.

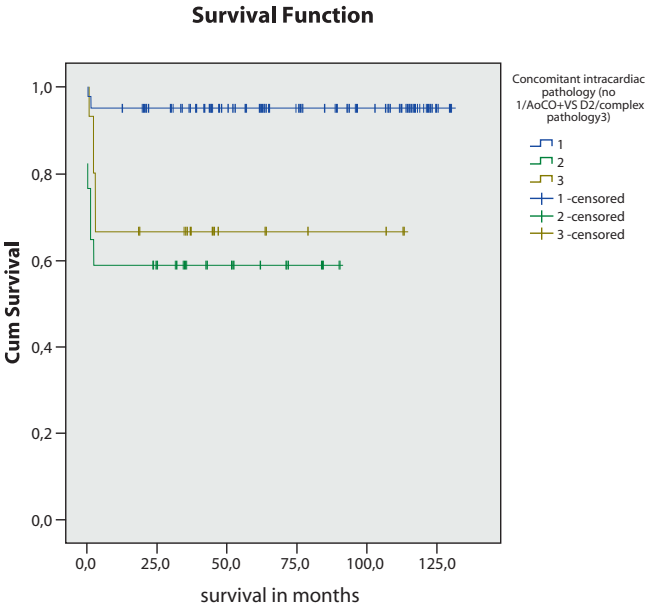
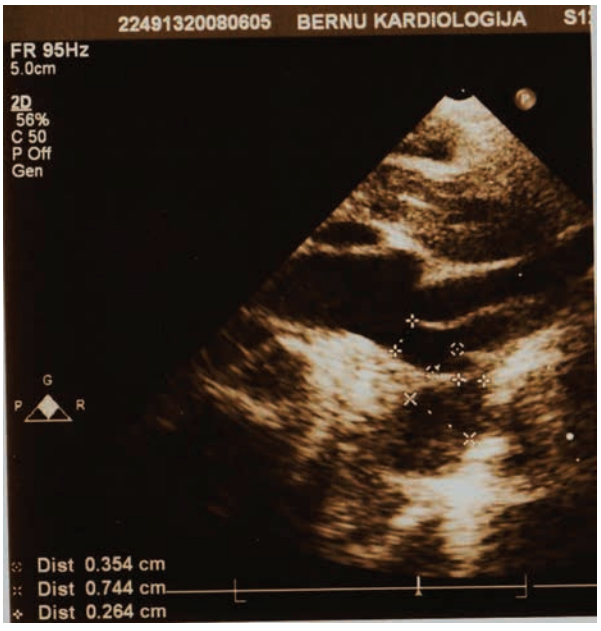
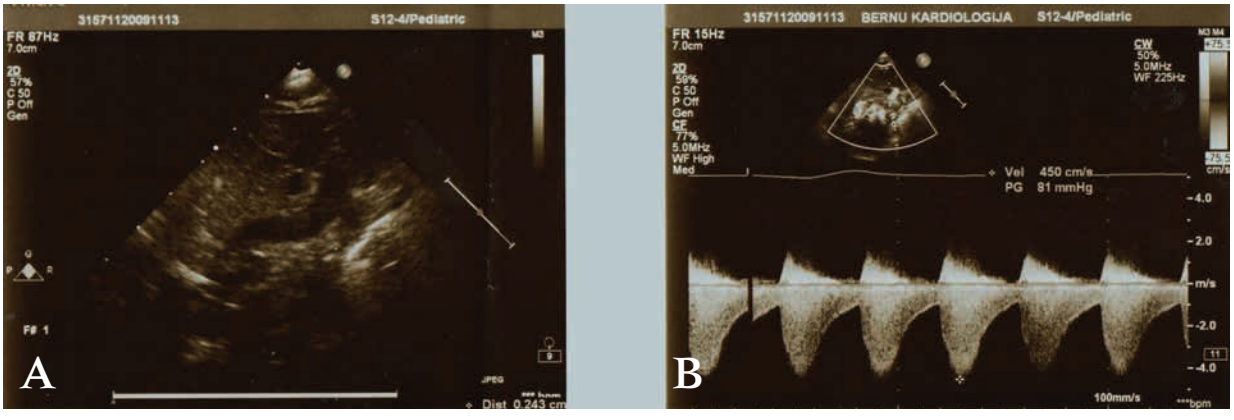


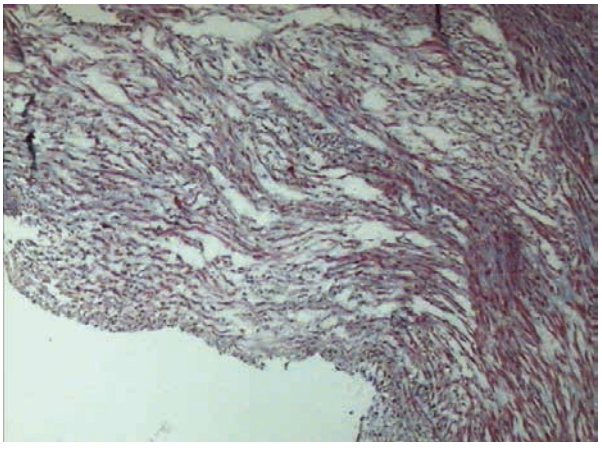
Fig. 4. Survival of patients with and without concomitant intracardiac pathology.



Picture 1. Echoracriography: neonate with infantile aortic coarctation and hypoplastic transverse aortic arch.



Picture 2. Echocardiography of severe AoCo(suprasternal long axis view) A, Continuous-wave Doppler through the aortic isthmus of a patient with severe AoCo (high velocity systolic amplitude(4,5m/s) with continuous antegrade flow throughout diastole B.



Picture 3. AoCo with fibrointimal thickening (Masson`s trichrome stain).

ORIGINAL ARTICLE

The Detection of Beta-Herpesvirus Infection in Patients undergoing Reconstructive Flap Surgeries and Its Association with the nearest Postoperative Period Course

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Summary

Introduction. Reconstructive free flap surgery is a complex method of wound closure for large wounds not amenable to linear (primary) closure. It involves the transfer of free tissue (muscle, bone or a combination) to a site of tissue loss where its circulation is restored via microvascular anastomoses. Usually patients requiring reconstructive flap surgery are in the working age without significant co-morbidities. Considerable differences of clinical course after the trauma and in the postoperative period after free flap surgery such as surgical outcome, complication rate and duration, have been observed. Beta-herpesviruses 6 (HHV-6) and beta-herpesviruses 7 (HHV-7) are ubiquitous immunomodulating viruses after primary infection infecting individuals persistently throughout the life. The stimuli for reactivation of these viruses are uncharacterized, but are likely to include immunosupression. Anaesthesia proves to be an essential factor suppressing the immune system, in particular cell-mediated immunity, in the post-operative period. Anaesthesia-associated immunomodulating and intensification of immunosuppressive effect by beta-herpesviruses activation can increase susceptibility of patients to other infections such as bacterial, fungal infections and provoke postoperative complications.

The aim of the study. To investigate the presence of HHV-6 and HHV-7 in patients before prolonged reconstructive flap surgery and effect of prolonged reconstructive flap surgery upon general and regional anaesthesia on activation of HHV-6 and HHV-7 infections and how this activation is associated with postoperative period course.

Materials and methods. 38 patients after long lasting reconstructive microsurgical flap surgery procedures were enrolled in this investigation. For 17 patients general anaesthesia (GA) and for 21 patients regional anaesthesia (RA) was used. The markers of latent and active HHV-6 and HHV-7 infection were detected. Duration of postoperative period, time spent in the intensive care units (ICU), and number of repeated surgeries after the trauma, therapy outcome in relation to the anaesthesia method and activation of β-herpesviruses were assessed.

Results. Before the surgery latent HHV-6 was revealed in 8 patients (GA) and in 6 patients (RA), active HHV-6 in 2 patients (GA), in 1patients (RA). Latent HHV-7 was revealed in 11 patients (GA), in 13 patients (RA) and active HHV-7 - in 4 patients (GA) and in 4 patients (RA). In 5 patients (GA), in 3 patients (RA) concurrent latent HHV-6/ HHV-7 infection was found, in patient 1 (GA) it was active. After the surgery reactivation of HHV-6 was detected in 1 patient (GA), in (RA) no cases of activation. Reactivation of HHV-7 was detected in 4 patients (GA) in 1 patient (RA). Simultaneous reactivation of HHV-6/HHV-7 was detected in 1 patient (GA). Postoperatively (GA): 6 cases of unfavourable surgery (4 -surgical site infection (SSI), 2 - flap ischemia) were observed. Postoperatively (RA): 2 cases of SSI were observed. In 30 (78.94%) patients of both groups (RA and GA) to whom reactivation of the viruses after surgery was not revealed, 5 (17.7%) patients had unfavourable surgeries.

In 5 (20%) patients to whom activation of the viruses after the surgery was revealed, unfavourable surgery was in 1 patient. In 2 patients (GA) with active HHV-6 viral infection already before surgery had SSI.

Conclusions. To the best of our knowledge, this is the first study documenting the presence and activation of HHV-6 and HHV-7 infection in patients undergoing prolonged reconstructive surgery. Despite the limited number of patients our study results suggests that the presence of HHV-6 and HHV-7 infection in our study group was significantly high. Reactivation of HHV-6 and HHV-7 infection is more frequent in patients to whom general anaesthesia is applied. Our results suggesting that reactivation of HHV-6 and HHV-7 infection is possibly related to longer and more complicated postoperative period with a worse clinical outcome.

Key words: anaesthesia, HHV-6, HHV-7, postoperative period.

INTRODUCTION

Reconstructive free flap surgery is a complex method of wound closure for large wounds not amenable to linear (primary) closure. It involves the transfer of free tissue (muscle, bone or a combination) to a site of tissue loss where its circulation is restored via microvascular anastomoses. Flaps may be pedicled or free. Pedicled flaps keep their primary vascular supply while free flaps

have their circulation detached and reanastomosed distantly.

Appropriate anaesthetic management for reconstructive flap surgery consists of maintenance of high cardiac output, normal arterial blood pressure (systolic > 100 mm Hg), low systemic vascular resistance, normothermia, effective analgesia, high urine output (> 1-2 ml/kg/h), low viscosity (haematocrit at 30%), monitoring of blood flow in flap (Doppler postoperatively). Balanced general anaesthesia, good analgesia and normothermia provide vasodilatation. A regional anaesthetic technique is preferred to cover the free flap recipient site (Quinlan J, Lodi O., 2009).

Usually patients requiring reconstructive flap surgery are in the working age without significant co-morbidities. Regardless of the young age and adequate previous medical condition of patients, considerable differences of clinical course after the trauma and the postoperative period after free flap surgery such as surgical outcome, complication rate and duration, have been observed. Beta-herpesviruses HHV-6 and HHV-7 are ubiquitous immunomodulating viruses after primary infection infecting individuals persistently throughout the life. The stimuli for reactivation of these viruses are uncharacterized, but are likely to include immunosuppression. Clinical and experimental evidence indicates that HHV-6 and HHV-7 can interfere with the function of the host immune system through a variety of mechanisms. HHV-6 and HHV-7 infect cells of immune system as an integral part of their life cycle. By infecting immune cells of both innate and adaptive immune responses, these two viruses have the potential to impair host defense system seriously. Human herpesvirus 6 (HHV-6) and human herpesvirus 7 (HHV-7) are similar and share common biological properties and nucleotide sequences and are classified as members of the roseolovirus genus of the beta-herpesvirus subfamily. Most HHV-7 protein sequences share 30% to 60% amino acid sequence identity with their HHV-6 counterparts (Yamanishi *et al.*, 2007). All of the roseoloviruses infect T lymphocytes in vivo and in vitro, cause similar damage to infected cells, and have overlapping but distinct disease spectra.

Primary infection with HHV-6 is the major cause of roseola infantum (also known as roseola, exanthem subitum, 3-day-fever, or sixth disease), a febrile rash illness common in early childhood (Braun *et al.*, 1997; Yamanishi *et al.*, 2007); primary HHV-7 infections can also cause roseola. Roseoloviruses can affect the central nervous system; patients with HHV-6 primary infection sometimes develop seizures or convulsions.

HHV-6 infect various types of neural cells and in some studies have been associated with demyelinating diseases, including multiple sclerosis (MS) and progressive multifocal leukoencephalopathy (Caserta *et al.*, 2001; Tyler, 2003). HHV-6 associated pneumonia, encephalitis, hepatitis, and hematopoietic stem cell suppression have been observed after stem cell transplantation (Clark and Griffiths, 2003; Ljungman, 2002; Yoshikawa, 2003). In addition, HHV-6 and HHV-7 immune suppressive activities may also indirectly contribute to fungal, bacterial, or cytomegalovirus (CMV) infections (Boeckh and Nichols, 2003; Ljungman, 2002).

A role for HHV-6 infections in the progression of HIV disease has been debated (Braun *et al.*, 1997; Caserta *et al.*, 2001; Clark and Griffiths, 2003). Continuing exploration there was found reactivation of herpesviruses in children with acute appendicitis (Katzoli *et al.*, 2009).

HHV-6 and HHV-7 may have several indirect impacts on transplant recipients, including increased risk of infections and rejection episodes (Shiley and Blumberg 2010).

There is a strong and specific relationship between HHV-6 infection and the use of cord blood cells (Chevallier et al.2010). Study done by (Kirac et al.2008) indicated a possible role for HHV-6 and especially HHV-7 in a pathogenesis of Pityriasis rosea.

The presence of concurrent HHV-6 and HHV-7 DNA in the plasma is an unfavorable prognostic factor (Chapenko et al.2009).

Autoimmune connective tissue diseases (ACTD) encompass many diseases, including systemic sclerosis or scleroderma, systemic lupus erythematosus, discoid lupus erythematosus, dermatomyositis, vasculitis and other conditions causing chronic inflammation that can affect many organs and systems. The frequent reactivation of HHV-6 in scleroderma and other ACTD, especially when active suggests that HHV-6 may play a role in the pathogenesis of these diseases (Broccolo et al.2009).

The consequences of HHV-6 and HHV-7 infections after transplantation are classified into direct and indirect effects. Overt clinical disease directly due to HHV-6 has been estimated to occur in less than 1% of patients. Most of these have been due to HHV-6B, although a few cases of HHV-6A associated disease have been reported. In this setting, HHV-6 may be manifested as a febrile syndrome accompanied by some degree of bone marrow suppression, an illness similar to CMV syndrome. Reactivation of HHV-6 and HHV-7 in renal graft recipients is a risk factor for development of chronic allograft nephropathy (Chapenko et al. 2009).

HHV-7 infection is not well documented, although a few cases of febrile syndromes and acute myelitis have been described in transplant recipients (Razonable 2009)

The most common outcome of infection with these viruses is not disease itself, but a lifelong dynamic biological interaction between the virus and host that seldom manifests as disease.

These viruses can be reactivated in immunosuppressed states resulting in the development of direct pathological lesions. It is also possible that these viruses may indirectly contribute to the activation of other infectious processes by inhibiting the immune system.

Anaesthesia proves to be an essential factor suppressing the immune system, in particular cell-mediated immunity, in the post-operative period. Anaesthesia-associated immunomodulation and intensification of immunosuppressive effect by beta-herpesviruses

activation can increase susceptibility of patients to other infections such as bacterial, fungal infections and provoke postoperative complications, e.g., wound-healing disturbances and infections, leading to sepsis, followed by multiple organ failure and potential lethal outcome (Homburger and Meiler, 2006; Mamaja et al., 2008).

The aforementioned differences during postoperative period course have stimulated research on how the prolonged reconstructive flap surgery and anaesthesia technique might impact reactivation of these viruses and how activation of these viruses is associated with nearest postoperative period course.

AIM OF THE STUDY

This study aimed to investigate the presence of human herpes virus 6 (HHV-6) and human herpes virus 7 (HHV-7) in patients before prolonged reconstructive flap surgery and effect of prolonged reconstructive flap surgery upon general and regional anaesthesia on activation of human herpes virus 6 (HHV-6) and human herpes virus 7 (HHV-7) infections and how this activation is associated with postoperative period course.

MATERIALS AND METHODS

A retrospective analysis was done for 38 patients (aged 5-65) who underwent long lasting (average 5.7h) reconstructive flap surgery procedures in The Centre of Plastic and Reconstructive Microsurgery of Latvia. Cohort was established with the approval of the Ethics Committee of the Riga Stradiņš University and all participants gave their informed consent prior to the examination. Patients were split into two study groups according to anaesthesia method applied - general anaesthesia (GA) group and regional anaesthesia (RA) group.

For 17 patients general anaesthesia (GA) and for 21 patients regional anaesthesia (RA) was used. Patients were not randomized depending on anaesthesia method but surgical necessity, priority was given to regional anaesthesia.

Table 1. Patients' characteristics

	General (n=17)	Regional (n=21)
Age (yr)	37.58 (5-60)	41.68 (16-65)
Sex M/F	9/8	15/6
ASA I/II/III	3/14/1	5/15/1
No. of previous operations after trauma	2.75 (0-9)	1.28 (0-3)
Duration of reconstructive flap surgery (h)	5.92	5.58

Data are means or range

Table 2. Cause of primary defect

	General (n=17)	Regional (n=21)
Extensive Surgery (Breast Cancer)	5	0
Upper Extremity Trauma	3	5
Lower Extremity Trauma Congenital defects	6 3	16 0

Table 3. Reconstructive flap surgery procedures performed

Type of flap	Number of surgeries provided
1. Pedicle flaps	
• TRAM*	3
2. Free flaps	
• Muscular flaps	7
• Fasciculocutaneus flaps	11
• Osteocutaneus flaps	13
3. Toe transplantation	4

*Transverse rectus abdominus muscle

GA was performed: for induction midazolam (0.05 mg/kg), propofol (1.5-2 mg/kg), fentanyl (1.5 μg/kg), and cis-atracurium (0.2 mg/kg) was given to enable tracheal intubation. For maintenance of anaesthesia, isoflurane (minimal alveolar concentration (MAC) 0.5-1) and fentanyl 2 mcg/kg/h and cis-atracurium (0.03 mg/kg/h) via continuous infusion were given.

RA was performed with two methods simultaneously (spinal anaesthesia and brachial plexus block-supraclavicular, infraclavicular or axillar aproach). For brachial plexus block, bupivacaine (0.5%-20 ml) or levo-bupivacaine (0.5%-20 ml) in combination with lidocaine (1%-20 ml) and nerve stimulation was used. For spinal anaesthesia bupivacaine (0.5%-4 ml) and 25 μg Fentanyl or clonidine 15-30 μg as adjuvant at L2-L3 was used. All patients who received RA were sedated with midazolam.

Monitoring consisted of five leads electrocardiography (ECG), peripheral oxygen saturation (SaO_2), fractional inspired oxygen concentration (FiO_2), fractional expired carbon dioxide concentration ($FeCO_2$), minimal alveolar concentration (MAC) of volatile anaesthetic, core and peripheral temperature measurement, central venous pressure (CVP) and non-invasive arterial pressure. The depth of anaesthesia and sedation was monitored with the bispectral index (BIS) monitor. Urine output as indicator of volume status was monitored perioprativelly. Anti-coagulated peripheral blood samples for the detection of latent/persistent or active viral infection were collected from patients before the anaesthesia and reconstructive flap surgery and 10 days after the anaesthesia and surgery.

Nested polymerase chain reaction (nPCR) was used for the detection of HHV-6 and HHV-7 sequences in peripheral blood leukocytes (PBL) and plasma DNAs. The presence of viral sequences in PBL DNAs was a marker of latent/persistent viral infection and in plasma DNAs – of active viral infection (plasma viremia).
Duration of postoperative period, time spent in the intensive care units (ICU), number of repeated surgeries after the trauma, therapy outcome in relation to the anaesthesia method and activation of β-herpesviruses were assessed.

RESULTS

Before the surgery latent/persistent HHV-6 infection was revealed in 14 out of 38 patients (36.84%) (in 8 patients in GA group and in 6 - in RA group) and active HHV-6 infection - in 3 out of 38 patients (7.89%) (in 2 patients in GA group and in one - in RA group). In 21 out of 38 patients (55.27%) HHV-6 infection was not detected. At the same time latent/persistent HHV-7 infection was revealed in 24 out of 38 patients (60%) (in 11 patients in GA group and 13 - in RA group) and active HHV-7 - in 8 out of 38 patients (21%) (in 4 patients in GA group and in 4 - in RA group). In 6 out of 38 patients (15.79%) HHV-7 infection was not detected. In 8 patients (5 patients in GA group and 3 patients - in RA group) concurrent latent/persistent HHV-6/ HHV-7 infection was found and in one of them (in GA group) concurrent infection was active.

Table 4. Frequency of absent/latent/persistent and active HHV-6 and HHV-7 infection before and after anaesthesia and surgery

Anaesthesia		Absent/latent/ active viral infection (n)	
		HHV-6	HHV-7
Before the surgery	General (n=17)	7/8/2	2/11/4
	Regional (n=21)	14/6/1	4/13/4
After the surgery	General (n=17)	7/7/3	2/7/8
	Regional (n=21)	14/6/1	4/12/5

After the surgery latent/persistent HHV-6 infection was detected in 13 out of 38 patients (34.31%) (in 7 patients in GA group and in 6 - in RA group) but active HHV-6 infection - in 4 out of 38 patients (10.51%) (in 3 patients in GA group and in one - in RA group). 21 out of 38 patients (55.27%) was HHV-6 infection free. Whereas latent/persistent HHV-7 infection was revealed in 19 out of 38 patients (50%) (in 7 patients in GA group and 12 - in RA group) and active HHV-7 infection - in 12 out of 38 patients (31.57%) (in 8 patients in GA group and in 4 - in RA group), but 6 out of 38 patients (15.79%) were HHV-7 infection free. In 8 patients (in 4 patients in GA group and 4 patients in RA group) concurrent latent/persistent HHV-6/HHV-7 infection was detected but in one patient in GA group concurrent infection was active.
After long lasting reconstructive free flap surgery in one patient of the GA group reactivation of HHV-6 infection was detected in contrary to the RA group where no cases of reactivation were revealed. At the same time in 5 patients (4 patients in GA group and one - in RA group) reactivation of HHV-7 infection was detected. In one patient in GA group simultaneous activation of HHV-6 and HHV-7 was detected after the surgery whereas in RA group simultaneous activation of HHV-6 and HHV-7 was not revealed.

Table 5. The main characteristics of postoperative period

	General Anaesthesia	Regional Anaesthesia
All patients	**(n=17)**	**(n=21)**
Number of unfavourable surgeries	6	2
• Surgical site infections	4	2
• Flap ischemia	2	0
Number of repeated surgeries until second blood sample (n)	2.38±0.9	1.2±0.3
Time spent in ICU (days)	4.17±9.77	2
Duration of postoperative period (days)	23.76±20.75	11.5±8.76

Data are means and SD

In GA group 6 cases of unfavourable surgery results were observed. In 4 cases these were surgical site infection whereas in 2 cases - flap ischemia.
In patients of the RA group 2 cases of unfavourable surgery results were observed. Both cases were surgical site infection. The number of repeated surgeries following prolonged reconstructive surgery until second blood sample was 2.38±0.9 in patients of GA group and 1.2±0.3 in RA group. In GA group the postoperative period was significantly longer (23.76±20.75 days) in comparison with RA group (11.5±8.76 days). Patients from GA group after a long lasting reconstructive surgery spent on average 4.17±9.77 days also in ICU. For patients of RA group the need to treat patients in the ICU was shorter (2 days).

Table 6. Postoperative period course depending on the reactivation of viral infection

	General Anaesthesia	Regional Anaesthesia
In patients without reactivation	**(n=11)**	**(n=19)**
Number of unfavourable surgeries	4	1
• Surgical site infections	2	1
• Flap ischemia	2	0
Number of repeated surgeries until second blood sample (n)	2±2.7	2±2.7
Time spent in ICU (days)	4.46±11.14	0.16±0.7
Duration of postoperative period (days)	23.76±21.97	10.1±5.56
In patients with active viral infection before surgery	**(n=2)**	**(n=3)**
Number of unfavourable surgeries	1	1
• Surgical site infections	1	1
• Flap ischemia	0	0
Number of repeated surgeries until second blood sample (n)	0	2
Time spent in ICU (days)	0.5	0.66
Duration of postoperative period (days)	29.33	22
In patients with reactivation of one virus	**(n=3)**	**(n=1)**
Number of unfavourable surgeries	1	0
• Surgical site infections	1	0
• Flap ischemia	0	0
Number of repeated surgeries until second blood sample (n)	3.33	3
Time spent in ICU (days)	4.33	0
Duration of postoperative period (days)	29.3	10
In patients with reactivation of two viruses	**(n=1)**	**(n=0)**
Number of unfavourable surgeries	0	0
• Surgical site infections	0	0
• Flap ischemia	0	0
Number of repeated surgeries until second blood sample (n)	0	0
Time spent in ICU (days)	0	0
Duration of postoperative period (days)	7	0

Data are means and SD

In 30 (78.94%) patients, irrespective on anaesthesia method applied, reactivation of the viral infection after the reconstructive surgery was not revealed. In this group of patients 5 (17.7%) patients were followed with unfavourable surgeries. In 5 (20%) patients in whom activation of the viral infection after the surgery was revealed, unfavourable surgery was in 1 patient. Regardless of whether reactivation the activation took place or not, surgical outcome did not differ.

At the same time we observed relationship between active HHV-6 viral infection before reconstructive flap surgery and postoperative surgical site infection irrespective of the anesthesia technique applied.

DISCUSSION

A lot of interest has been concentrated on identifying the pathologic role of lymphotropic human herpesviruses HHV-6 and HHV-7 viruses in patients. The rationale is that all the other herpesviruses cause significant diseases in this group of patients. In addition to directly causing pathologic lesions, it is also possible that these viruses (HHV-6 and HHV-7) may indirectly contribute to other infectious pathogens by inhibiting the immune system (Griffiths, 2003).

Our current study is the first study documenting the presence and activation of HHV-6 and HHV-7 infection in patients undergoing long lasting reconstructive flap surgery (average 5, 7 h).

In the previous study conducted in Latvia and aimed to detect the blood-borne viral infections among apparently healthy Latvian blood donors, the following incidence of latent beta-herpesvirus infections is revealed: single infection by HHV-6, and HHV-7 is detected in 8.0%, and 43.3% of blood donors, respectively. (Kozireva et al., 2001).

In our patients after trauma and previous surgery and before reconstructive flap surgery and anaesthesia we have revealed significantly higher incidence of latent HHV-6 and HHV-7 viral infection. HHV-6 and HHV-7 is detected in 44.7%, and 84.1% patients, respectively.

Compared results from healthy Latvian blood donors and patients of our study before reconstructive flap surgery, which includes patients after severe trauma, we have revealed 5.5 times higher incidence of HHV-6 and 1.9 times higher incidence of HHV-7 in patients from our study.

In the previous study conducted in Latvia active infection of HHV-6 is not found, but active HHV-7 is present in 10.6% of the blood donors (Kozireva et al., 2001).

At the same time we have found active HHV-6 and HHV-7 viral infection before reconstructive flap surgery and anaesthesia in 7.9%, and 26.3% patients, respectively.

It could indicate for our study patients that and trauma and following surgeries could initiate reactivation of viral infection.

HHV-6 and HHV-7 can be reactivated in immunosuppressed states. HHV-6 also affects almost all the components of the immune system, including both innate and adaptive immune functions. HHV-6 replicates

in and kills CD4+ and CD8+ T-cells. HHV-6 viral envelope proteins inhibit T lymphocyte proliferation induced by phytohemagglutinin (PHA), IL-2, or antigens (Horvat *et al*., 1993). Interaction of inactivated HHV-6 viral particles with PBMC inhibits proliferation of both CD4+ and CD8+ T lymphocytes and their responses to IL-2. This defect is apparently due to induction of defective IL-2 receptors or defects in IL-2 induced signalling pathway in these cells, as exogenous IL-2 does not correct the HHV-6 induced proliferation defect (Flamand *et al*., 1995). In our study we observe, that patients with active HHV-6 infection before reconstructive flap surgery, have infectious complications, which could be related to imunomodulatory properties of HHV-6.

Numerous studies (Kurosawa S, 2008) have shown that recently, alongside with immune suppression caused by surgical stress, anaesthetics and analgesic agents commonly used in surgery and in intensive care may directly affect the functions of immune-competent cells. Since the reactivation of HHV-6 and HHV-7 viral infection is more pronounced in patients of general anaesthesia group, our data are consistent with the previous studies that general anaesthesia does not suppress the surgical stress response, thus exacerbating postoperative immunosupression (Stevenson, 1990), (Lundy, 1978). Immunosupression is the main cause of the reactivation of HHV-6 and HHV-7 viral infection. Spinal anaesthesia results in less immunosupression, i.e. it maintains the number of Th1 cells, thus stimulating cell immunity. The effects are most pronounced in high risk patients undergoing procedures below the umbilicus (Liu, 1995). Serious disorder of the immunological system may cause complications, as there are disorders in wound healing, increased number of infections, inadequate response to stress, multiorganic suppression and increased incidence of metastases (Rosen, 1992). The data of our study shows that reconstructive flap surgery under general anaesthesia could significantly increase beta-herpesviruses activation frequency in comparison with long lasting reconstructive surgery with RA. Duration of postoperative period in general anaesthesia group is significantly longer in comparison with regional anaesthesia (23.7 and 11.5 days respectively) in spite of the same surgical injuries. Also the need for treatment in ICU is significantly longer (4.4 and 0.1 days respectively). This is due to higher number of unfavourable surgeries in general anaesthesia group.

Despite limited number of patients and low incidence of postoperative surgical site infection our study shows a significant relationship between active HHV-6 viral infection before reconstructive flap surgery and postoperative surgical site infection irrespective of the anesthesia technique applied. Both patients with active HHV-6 viral infection before surgery suffered from osteomielitis before reconstructive flap surgery and postoperative period course was with infectious complications.

In our opinion there could be a possible link between immunosupression due to anaesthesia and a possible reactivation of β-herpesviruses. General anaesthesia could lead to suppression of cellular immune response in patients with latent viral infection and significantly increase frequency of virus reactivation in comparison with regional anaesthesia. This may cause more difficult postoperative period and patients' recovery.

CONCLUSIONS

To the best of our knowledge, this is the first study documenting the presence and activation of HHV-6 and HHV-7 infection in patients undergoing prolonged reconstructive surgery.

Despite the limited number of patients our study results suggests that the presence of HHV-6 and HHV-7 infection in our study group was significantly high.

Reactivation of HHV-6 and HHV-7 infection is more frequent in patients to whom general anaesthesia is applied.

Our results suggesting that reactivation of HHV-6 and HHV-7 infection is possibly related to longer and more complicated postoperative period with a worse clinical outcome.

Conflict of interest: None

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ORIGINAL ARTICLE

The Use of Deceased Donors for Kidneys Transplantations

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Summary
Introduction. The lack of donor organs aimed at transplanting is a great problem all over the world. To solve the problem the criteria of selecting donors progressively expand including donors after cardiac death. Analysis performed in this report refers to the use of donors after the controlled cardiac death to transplant kidneys at the Latvian transplantation center.
Aim of the study. To clarify the renal transplant outcomes after transplantation from deceased donors of two types – donors after cardiac death and donors after brain death.
Materials and methods. Study included 351 consecutive primary renal transplantations from deceased donors procured in a single transplant center during the period since 2000 till 2005. Kidneys were recovered from 244 deceased donors at the age of 41.2 + 13.2 (8 - 68): from 68 – after cardiac death (DCD) and from 176 – after stating the brain death (DBD), (control group). Results of transplantations were compared regarding the type of deceased donors been used.
Results. The percentage of non functioning graft recovered from donors after cardiac death made up 1.8 % (at the rate of 1.6 % in a control group). Delayed graft function of transplants made up respectively 14.0% and 12.3%. Within a period of 5 years mortality in DCD group made up 15 % and in BDB group 12.3 %. Graft survival was respectively 76.7 % and 84.8% (p = 0,046).
Conclusion. Results of study show the efficiency of transplanting kidneys received from donors after cardiac death and brain death. Long term results in DCD donation should be improved by introduction of new technologies.
Key words: transplantation, DCD, kidney.

INTRODUCTION

The lack of donor organs aimed at transplanting is a great problem all over the world. To solve the problem the criteria of selecting donors progressively expand including donors after cardiac death (1). The use of such donors is related to warm ischemia prior to organs explantation, as well as with the lack of an adequate donor management, which may have a negative effect on the result of transplantation (2). Donors after cardiac death have been used in Latvia since 1992. Analysis performed in this report refers to the use of donors after the controlled cardiac death to transplant kidneys.

AIM OF THE STUDY

To clarify the renal transplant outcomes after transplantation from deceased donors of two types – donors after cardiac death and donors after brain death.

MATERIALS AND METHODS

Study included 351 consecutive primary renal transplantations from deceased donors procured in a single transplant center during the period since 2000 till 2005 (tab. 1). Kidneys were recovered from 244 deceased donors at the age of 41.2 $\pm$ 13.2 (8 - 68): from 68 – after cardiac death (DCD) and from 176 – after stating the brain death (DBD), (control group). Donors of 3rd and 4th category were used after cardiac death pursuant to the Maastricht classification (3rd category – expected cardiac arrest, 4th –cardiac arrest in the process of stating the brain death diagnosis (3). It passed about 5-15 minutes from the time of stating a stable cardiac arrest and till the beginning of organs perfusion. Brain death was stated on the basis of the generally accepted clinical criteria. Conservation in-situ was performed by Custodiol. The mean cold ischemia time (CIT) was 16 $\pm$ 5 hours.

Primary kidney transplantation was performed in 351 recipients with chronic kidney disease at the terminal stage. The basic diseases the recipients suffered from were chronic glomerulonephritis (45%), chronic interstitial nephritis (20%), diabetic nephropathy (18%), kidney polycystos (9%). The percentage of pre-reactive antibodies (PRA) was within the range of 0-20%. At 107 cases kidneys for transplantation were used from donors after cardiac death and at 244 cases – having stated the brain death. Donor-recipient pairs were selected according to ABO compatibility and at a negative cross matching. Induction immunosuppression was performed by Basiliximab and steroid pulse, maintenance - by Sandimmune Neorale, Mycophenolate Mofetil and Prednisolone. Acute rejection was treated with the pulse dosage of Methylprednosolone, and in the case of steroid resistant rejection – with antithymocyte globulin.

SPSS 13.0 (SPSS Inc.) program was used for statistical analysis. χ^2 test was used for nonparametric values; Caplan Meyer diagram was used to calculate the survival.

Table 1. Demographical factors.

Factor	Results
Total number of deceased donors, n DCD/DBD, n Age of deceased donors, years DCD/DBD, years	244 68 / 176 41.2 ± 13.2 42, 7 ± 11,5 / 39,3 ± 13,5
Total number of transplantation, n DCD/DBD, n Age of recipients DCD/DBD, years	351 107 / 244 45,6 ± 14,5 / 44,2 ± 15,0
Diagnoses: Chronic glomerulonephritis Chronic interstitial nephritis Diabetic nephropathy Kidney polycystos Others	 45 % 20 % 18 % 9 % 8 %
Mean cold ischemia time	16 ± 5 hours

RESULTS

During the past year abundance of DCD increased and exceeded 40% of the total number of donors. The age structure of the donors and the recipients of both groups did not differ from each other. The percentage of non functioning grafts received from donors after cardiac death made up 1.8 % (at the rate of 1.6 % in a control group). Delayed function of transplants was observed in respectively 14.0% and 12.3%. There was no essential distinction in the development of acute rejection (respectively 30.8% and 28.6%). The difference was observed when providing follow-up studies: graft survival was respectively 76.7 % and 84.8% ($p = 0,046$). Within a period of 5 years patient lethality in DCD group made up 15 % and in BDB group 12.3 % (Fig. 1).

Table 2. Results of the primary renal transplantions from donors after cardiac death and after stating the brain death (2000-2005).

Factor \Type of donor	DCD	DBD	*p*
NFG*	2 (1.8 %)	4 (1.6%)	NS
DGF**	15 (1.0%)	30 (12.3%)	NS
AR***	33 (30.8%)	70 (28.6%)	NS
Graft loss****	25 (23.3 %)	37 (15.2%)	0,046
Patient lethality	16 (15.0 %)	30 (12.3 %)	NS

* NFG – non-functioning graft, ** DGF - delayed graft function, *** AR - acute rejection, **** death-censored graft loss.

DISCUSSION

Regarding the fact that early results did not differ in both groups, prove a negative effect of brain death on the function of transplanted organs and the lack thereof as such at donors after cardiac death compensates a negative effect of warm ischemia (5). The results of long-term observations in our transplantation center have shown the difference in graft survival. One of the reasons of the difference in survival is by far related to warm ischemia of a transplant and the subsequent accelerated development of fibrotic changes (6). One of the ways for at least partial possible improvement of this index is to apply new technologies such as a perfusion machine (7-8). Recovery of organs (kidneys, liver, and lungs) at donors after cardiac death may be a perspective and steady-state method and the development of the use of such donors may considerably increase the number of transplants.

A critical problem of receiving organs after cardiac death is the maximum possible cutting of the time of safe warm ischemia. Efforts are made all over the world to maximum reduce the time of thermal ischemia and to start organs perfusion immediately after stating a sustainable asistoly. A series of methods have been offered with this aim: organs cooling by way of the perfusion of cooled preserving liquid through premortum cannulated femoral vessels, applying reperfusion technique for normothermic extracorporeal oxygenation, etc. (9).

Legislation is the other opportunity. There is presumed consent of assent applicable in Latvia and the opportunity for expressing own opinion via the State Population Register. The use of these opportunities enables timely preparing for organs recovery while waiting the possible stable asystoly in perspective deceased donors.

CONCLUSIONS

Study show the efficiency of transplanting kidneys received from donors after cardiac death and brain death. Long term results in DCD donation should be improved by introduction of new technologies.

Conflict of interest: None

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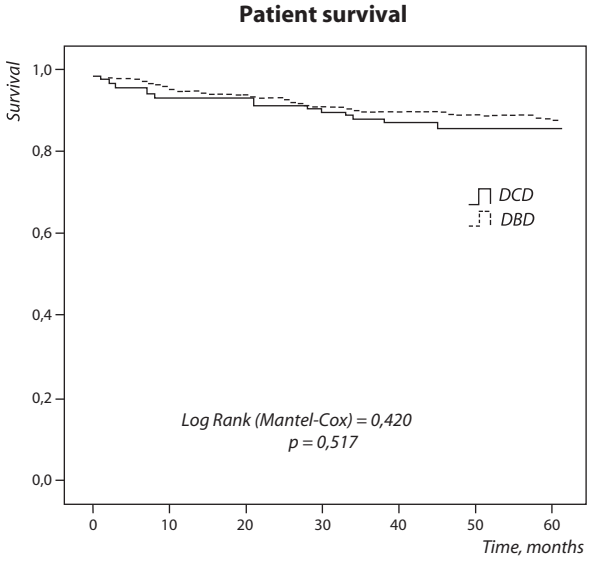

Fig. 1. 5-year patient survival

PROBLEM-SOLVING ARTICLE

Pathological Features of *BRCA1/BRCA2* Mutation-Associated Breast Cancer: Implications for Diagnostics and Treatment

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Summary
BRCA1 and *BRCA2* gene mutations are responsible for significant hereditary breast cancer burden. *BRCA1/2* mutation-associated breast cancers (further *BRCA1* or *BRCA2* cancers) are distinctive not only by family history but also by the biological features of the tumour influencing both diagnostic possibilities and response to different treatment modalities. Distinctive morphology and immunohistochemical phenotype of hereditary breast cancers may help to identify patients who are likely to carry germ line mutations in *BRCA1* or *BRCA2* gene. The efficacy of specific treatment options can be predicted as well. Additionally, *BRCA1* carcinomas have different histopathological manifestations from *BRCA2* cancers. The cellular and molecular characteristics of *BRCA1/2* breast cancer can explain the clinical data and provide prognostic and predictive information. Here, we discuss the peculiarities of breast cancer in *BRCA1/2* mutation carriers having significant implications in the diagnostics, surgical approach and overall planning of treatment.
Key words: breast cancer, *BRCA1*, *BRCA2.*

INTRODUCTION

Mutations in the *BRCA1* and *BRCA2* genes are responsible for significant hereditary breast and ovarian cancer burden. The cancers arising in *BRCA1/2* mutation carriers (further designated in short as *BRCA1* cancer, *BRCA2* cancer or *BRCA1/2* cancer, respectively) represent complex medical care problems due to peculiarities in the diagnostics, surgical approach and chemotherapy, as well as to the necessity to lower the overall cancer risk in a frequently young patient in the childbearing age. The studies of best prophylactic and treatment options have highlighted novel data. In order to implement these findings deliberately in evidence-based everyday practice (Table 1), Virchow's principle to study the cell could be a reasonable way.

The studies of *BRCA1/2* mutation-associated breast cancer would have not only theoretical value in the research of cancerogenesis but also practical importance. Breast cancer is the most frequent malignant tumour in female both in Latvia and European Union (6, 16). There are approximately a thousand new cases of breast cancer in Latvia each year (data by the Central Bureau of Statistics of Latvia), and at least 2-5% could be related to *BRCA* mutation (10, 22). Reasonable rate of *BRCA1* mutations has been demonstrated in Latvia as well (21, 43). Therefore the considered problems are important in Latvia, and even more so in the present tight financial situation.

BRCA1/2 gene function is essential for the repair of DNA double-strand breaks through the process of homologous recombination (15). The lack of *BRCA1/2* gene product influences the intracellular events and may become the treatment basis.

High histological grade is one of the most stable features of *BRCA1* breast cancer. The proportion of high-grade (G3) carcinomas in *BRCA1* breast cancers ranges 66-85% in different studies, while in sporadic age-matched breast cancer group the proportion is 36-49% (4, 27, 33, 44). Breast cancers in patients with *BRCA1* mutations show higher mitotic counts, a greater degree of nuclear pleomorphism and less tubule formation (Fig.1) than sporadic age-matched breast cancer controls (27, 34). *BRCA2* mutation-associated tumours tend to be of moderate (G2) or high (G3) grade, and to have higher overall grade than sporadic controls (4, 27, 33). *BRCA2* cancers show less tubule formation, but pleomorphism and mitotic counts are similar to sporadic cases (27). As in general population, invasive ductal carcinoma is the most frequent histological type in *BRCA1/2* mutation carriers. *BRCA1* mutation-associated tumour group show higher incidence of medullary carcinoma (7-19%) than in *BRCA2* mutation carriers (0-3%) and non-carriers (0-3%) as described (13, 14, 27, 44). Medullary carcinoma is defined by syncytial growth, relatively circumscribed margins, marked or moderate lymphoplasmacytic infiltration, pleomorphic nuclei with high mitotic rate and absence of tubule formation. Areas of necrosis can be present. The pushing margins (Fig.2), reactive infiltration and necrosis are frequently exhibited in the *BRCA1* cancers independently of histological type, but cancers associated with *BRCA2* mutations show pushing margins as the only different feature from sporadic cases (1, 5, 8).

Invasive lobular carcinoma accounts for 5-25% of all breast cancers (25), and occurrence in hereditary cancer group is similar (27). However, Armes *et al.*,

1998 found that pleomorphic lobular carcinomas were more common in *BRCA2* mutation carriers (1). In addition, Marcus *et al* reported a higher proportion of *BRCA2* tumours belonging to a „tubular lobular group", including invasive lobular, tubular and cribriform carcinomas (30). Whereas, Lakhani *et al* found that tubular carcinoma was less common in *BRCA2* mutation carriers than *BRCA1* mutation carriers and non-carriers (27).

It was previously reported that *BRCA1/2* cancers may have a different way of tumorigenesis, progress more rapidly and may lack preinvasive lesions. Ductal or lobular carcinoma *in situ* adjacent to *BRCA1/2* mutation-associated invasive breast cancer was not always considered as a part of hereditary tumour spectrum (41). In addition, *in situ* component was less commonly found around invasive *BRCA1* tumours (27, 34). Conversely, in more recent studies the incidence of ductal carcinoma *in situ* (DCIS) is similar in *BRCA1/2*-tumours compared with sporadic controls, and authors suggest that DCIS is a part of the *BRCA1/2* cancer syndromes (3, 26). Therefore, genetic *BRCA1/2* testing should be considered for patients with preinvasive breast lesions as well.

Oestrogen, progesterone and HER-2 receptor negativity is described as a typical feature of *BRCA1* breast cancer. Negative expression of oestrogen receptors (ER) is present between 58-90% of *BRCA1* breast tumours compared with 23-35% in controls (4, 13, 17, 29, 34, 44) although relationship between ER status in *BRCA1/2* cancer and patient age, tumour grade and histological type is established. *BRCA1* mutation carrying patients in menopause are more likely to develop ER-positive breast cancer (12, 17, 44). Analysing G3 group, *BRCA1* cancers are 4.8 times more likely to be ER negative (Fig.3) than tumours in non-carriers (17). Between 58 and 79% of *BRCA1* carcinomas is reported to be PR negative in different series compared with 32-41% in sporadic cases (4, 29, 34, 44). In *BRCA2* tumours, in contrast to *BRCA1* tumours, frequency of ER and PR expression is similar to that in sporadic breast tumours (4, 17, 29, 34). The amount of ER positive cases in *BRCA2* tumours decreases with the increasing age. Furthermore, *BRCA2* carriers with G2 or G3 cancer are more likely to have ER positive cancer than non-carriers (17).

Although *BRCA1* mutation-associated breast cancers are mostly oestrogen receptor negative, tamoxifen or ovarectomy reduce breast cancer risk in mutation carriers. Tamoxifen has reduced risk of contralateral breast cancer by 30-40% in patients who receive it as treatment for the first breast cancer (24, 31). Although this level of protection is similar to the general population, the group of patients is highly selected as most *BRCA1* cancers have no indications for tamoxifen due to triple negativity. Oophorectomy can provide 50% risk reduction (24). The seeming controversy could be based on the complex relationship between *BRCA1* and oestrogen receptor alpha (22). Normally, *BRCA1* levels increase after oestradiol stimulation (22). This complex interaction possibly involves oestrogen-responsive elements in *BRCA1* promoter as well as indirect mechanisms. It may explain the tissue specifity of *BRCA1* mutation-related tumours. In turn, *BRCA1* inhibits oestrogen receptor alpha-mediated transactivation after oestrogen stimulation limiting oestrogen-dependant cellular proliferation. *BRCA1* can also transcriptionally induce oestrogen receptor alpha mRNA expression (22). HER-2 protein is over-expressed in 26% of all breast cancer cases and is classically associated with aneuploidy, high rate of proliferation and high histological or nuclear grade (38). These three features are encountered more frequently in *BRCA1* tumours. Despite this association, a significantly lower incidence of over-expression was observed among the *BRCA1* mutation-associated cases (2, 29). The rate of HER-2 over-expression can be as low as 3.6% (10). Although some authors found no differences in the expression of HER-2 between *BRCA1* related cancers and sporadic cases (4, 13, 34, 44), the possibilities of targeted anti-HER-2 therapy seem to be limited in *BRCA1* cancers. Data on HER-2 expression in *BRCA2* tumours also are variable. Some series found no difference in the expression of HER-2 in *BRCA2* and sporadic breast tumours (2, 4, 13), but some series revealed lower frequency of HER-2 expression in *BRCA2* tumours (29, 34).

The proliferation rate, evaluated by Ki-67, shows a consistently higher level (Fig.4) in *BRCA1* mutation-related breast cancers compared to sporadic cases (2, 34, 44). Among *BRCA1* mutation carriers, age-related differences were observed in the Ki-67 expression: the proliferation was higher in cases diagnosed before 50 years of age (44). Proliferation rate in *BRCA2* cancers is reported as medium in comparison with high in *BRCA1* tumours and very low in non-*BRCA1/2* tumours (34), or lower than in *BRCA1* cancers and without difference from sporadic cases (2).

E-cadherin (E-Cad) is transmembrane cell-cell adhesion receptor, which is present in epithelial breast cells. Expression of E-Cad is strongly associated with histological type and grade. Lobular carcinomas lack E-Cad expression. Most ductal carcinomas express E-Cad, but decrease of E-Cad expression is a frequent finding in high grade tumours (19). In addition, reduction of E-Cad expression is associated with a lack of steroid hormone receptors (32). Taking into account the morphological characteristics of *BRCA1* mutation-associated tumours, namely, ER and PR negativity and G3, more cases with reduced E-Cad expression would be expected in this group. Armes *et al.*, 1999 did not find significant difference of E-Cad expression (Fig.5) in breast tumours between *BRCA1*-, *BRCA2*- or non-mutation carriers. Palacios *et al.*, 2003 reported maintained E-Cad expression in the most cases of *BRCA2* cancers.

The analysis of the histopathological and immunohistochemical features of tumours arising in *BRCA1* mutation carriers has revealed that familial *BRCA1* mutation-associated tumours are characterised by a lot of histopathological features that belong to basal-like breast cancers. Similarities with basal-like phenotype at the immunohistochemical level are

striking: the vast majority of *BRCA1* mutation cancers lack ER, PR and HER-2 expression, frequently show p53 protein expression and high proliferation (1, 29, 33). Subsequent immunohistochemical studies have confirmed that a high proportion of *BRCA1* carcinomas, but only a small percentage of *BRCA2* carcinomas, expressed basal markers. *BRCA2* tumours are often of luminal phenotype and have features that are similar to those found in breast cancers from age-matched sporadic controls (45). Basal markers including cytokeratin (CK) 5/6 (18, 28, 35), CK14, CK17, osteonectin (28), P-cadherin (34) and epidermal growth factor receptor (EGFR) (28, 42) are more common in *BRCA1* tumours than in sporadic breast cancer cases. Collins *et al* mentioned that the expression of basal cytokeratins (Fig.6) and/or EGFR in triple-negative (ER-negative, PR-negative, HER2 not over-expressed) breast cancers allow to identify basal–like phenotype, but these markers alone are insufficient to reveal *BRCA1* mutation carriers (9).

Treatment of early-stage breast cancer includes surgery with or without radiotherapy and adjuvant systemic treatment. Breast-conserving therapy (BCT) is generally acceptable for early stage tumours, but it is questionable for *BRCA1/2* mutation carriers due to higher risk of ipsilateral breast tumour recurrence (IBTR) after BCT and higher risk of contralateral breast cancer (CBC) compared with patients who have sporadic breast cancer (20). *BRCA1/2* mutation carriers treated with breast conservation surgery may reduce IBTR by undergoing oophorectomy and decrease CBC risk by using tamoxifen (37). In addition, IBTR after BCT in *BRCA1/2* mutation carriers was not statistically significantly different by use of adjuvant radiotherapy or not (37). These data suggest that a more extensive surgical procedure than BCT could be performed and tamoxifen, despite of ER negativity, could be considered for *BRCA1/2* breast cancers in order to reduce the risk of breast cancer recurrence and CBC risk respectively.

Prophylactic bilateral salpingo-oophorectomy (PBSO) is currently the widely accepted preventive choice for *BRCA1/2* mutation carriers to reduce risk of both ovarian cancer and CBC. Laparoscopic PBSO should be a surgical option for *BRCA1/2* mutation carriers because of low morbidity and minimal-access surgery, and is strongly advised after age 35 or after childbearing. The most effective cancer preventive strategy is PBSO along with prophylactic mastectomy, but it is associated with a number of disadvantages of invasiveness, non-reversibility and surgical morbidity (40).

Aromatase inhibitors, that seem to surpass tamoxifen in increased efficacy and reduced toxicity, are effective only in the treatment of ER-positive breast cancers in postmenopausal women (23). Aromatase inhibitors may be choice for *BRCA1/BRCA2* carriers for CBC prevention who opt to have PBSO and breast surveillance.

Triple-negative and/or basal-like breast cancers has limited treatment options, as these cancers are resistant to existing targeted therapies, i.e., endocrine treatment and trastuzumab, leaving chemotherapy as the mainstay of treatment. There are no specific treatment guidelines for this subgroup, and prognosis is poor despite responding to conventional neoadjuvant and adjuvant chemotherapy regimens (7). Trials in mouse *BRCA1* tumours indicate that these tumours may be less sensitive to taxanes (39). However, the high incidence of EGFR reported in *BRCA1* cancers (42) may predict good response to anti-EGFR monoclonal antibodies and EGFR tyrosine kinase inhibitors.

Cisplatin induces the formation of cross linked DNA adducts between and within the strands. Cells possessing functional *BRCA* protein can repair the cisplatin-induced DNA damage by homologous recombination. *BRCA1* deficient cells are sensitive to cisplatin. However, resistance can develop, partially due to secondary *BRCA1* mutations restoring its function (15).

The recent discovery that poly(ADP-ribose)polymerase-1 (PARP-1) inhibitors kill *BRCA1/2* deficient cells with a high level of specifity opens up a potential therapy which looks very hopeful for *BRCA1/2* cancer patients. It is suggested that PARP inhibitors target DNA repair pathway and *BRCA* deficient cancer cells lack proteins necessary for DNA repair by homologous recombination. PARP-1 is a nuclear enzyme that participates in the DNA damage repair by base excision single strand repair pathway. If PARP-1 is blocked, DNA damage can be repaired by homologous recombination but this mechanism is primarily deficient in *BRCA1* mutant cells. Thus, upon the conditions of double inhibition, the DNA repair either is impossible or has to use less reliable pathways as non-homologous end joining or single strand annealing (15) leading to accumulation of defects until the cell dies. However, low PARP protein expression in *BRCA1* cancers was observed more often than in non-carriers, which may influence treatment results (11) therefore immunohistochemical evaluation of the relevant protein level could be suggested.

Predictive immunohistochemical profiling of *BRCA1* mutation-associated breast cancer has been carried out. PARP-1 immunoreactivity was found in 81.9% of *BRCA1* cancers, EGFR – in 43.6%, CD117 – in 14.7%, but 8.2% were negative for all studied predictive markers including also hormone receptors and HER-2 status by immunohistochemistry and fluorescent in situ hybridisation. In the result, the breast cancer patients with *BRCA1* mutation could possibly benefit from isolated or combined targeted therapies, using immunohistochemical evaluation for pharmacodiagnostics (10).

CONCLUSIONS

1. *BRCA1* mutation-associated breast cancers possess distinctive morphological and immunophenotypical features.
2. High grade and triple negative immunophenotype are characteristic for *BRCA1* mutation-associated breast cancer but these properties do not allow diagnosing the hereditary status with certainty. Elaboration of novel immunohistochemical markers would be helpful for cheap and fast screening.

1. The surgical treatment of breast cancer in *BRCA1* mutation carrier should be planned encountering the risk of recurrence and contralateral breast cancer.
2. The choice of screening method should encounter the gross and physical properties of *BRCA1/BRCA2* mutation associated breast cancer. More frequent presence of pushing margins can imitate benign process.
3. Prophylactic bilateral salpingo-oophorectomy and prophylactic bilateral mastectomy are feasible procedures with acceptable morbidity in selected high-risk patients.
4. Although there are no specific therapeutic guidelines for *BRCA1/2* breast cancers cisplatin may show higher efficacy in this group. The possibilities of targeted treatment range from limited in case of hormone receptors and anti-HER-2 to good in case of anti-PARP, c-kit or EGFR. The adjuvant and neoadjuvant treatment should be adjusted by mutation status. Novel chemotherapeutic agents that may prove effective in *BRCA1/2* mutation-associated breast cancer require further evaluation.

Conflict of interest: None

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Table 1. The features of *BRCA1/2* mutation-associated breast cancer and the relevant implications

Feature	Characteristics of *BRCA1/2* cancer	Clinical implications	Reference
Grade	High grade	Rapid growth	Lakhani *et al.*, 1997 Vaziri *et al.*, 2001 Palacios *et al.*, 2005 Atchley *et al.*, 2008
Mitotic activity	Higher	Rapid growth	Lakhani *et al.*, 1997 Palacios *et al.*, 2003
Histologic type	More frequent occurrence of medullary cancer	Misleading radiologic picture due to circumscribed growth Misleading FNA findings due to rich reactive inflammation	Lakhani *et al.*, 1997 Eisinger *et al.*, 1998 Vaziri *et al.*, 2001 Eerola *et al.*, 2005
Tumour margins	More frequently pushing margins	Misleading radiologic picture due to circumscribed growth	Armes *et al.*, 1998 Chappuis *et al.*, 2000 Bane *et al.*, 2007
Hormone receptors	Frequently negative in cancer	Lack of tamoxifen efficacy in treatment	Vaziri *et al.*, 2001 Lakhani *et al.*, 2002 Palacios *et al.*, 2003 Foulkes *et al.*, 2004 Eerola *et al.*, 2005 Atchley *et al.*, 2008
BRCA1/2 gene product interaction with estrogen receptor alpha	Preserved until mutation occurs in second *BRCA1/2* allele	Tamoxifen reduces contralateral breast cancer risk Ovarectomy reduces breast cancer risk	Narod et al., 2000 Guillem et al., 2006 Gorski et al., 2009
HER-2 protein over-expression	Rare	Rare indications for Trastuzumab	Armes *et al.*, 1999 Lakhani *et al.*, 2002 Domagala *et al.*, 2011
Proliferation rate	Higher	Faster growth Possible higher efficacy of chemotherapy	Armes *et al.*, 1999 Vaziri *et al.*, 2001 Palacios *et al.*, 2003
Molecular type	More frequent occurrence of triple negative breast cancer in *BRCA1* mutation carriers	Limited treatment options regarding Tamoxifen or Trastuzumab	Armes *et al.*, 1998 Lakhani *et al.*, 2002 Palacios *et al.*, 2005
DNA repair	Lack of homologous recombination	Cisplatin sensitivity PARP-1 inhibitor efficacy	Fasano and Muggia, 2009
EGFR status	Frequent expression	Good response to anti-EGFR monoclonal antibodies and EGFR tyrosine kinase inhibitors	van der Groep et al., 2004 Domagala et al., 2011

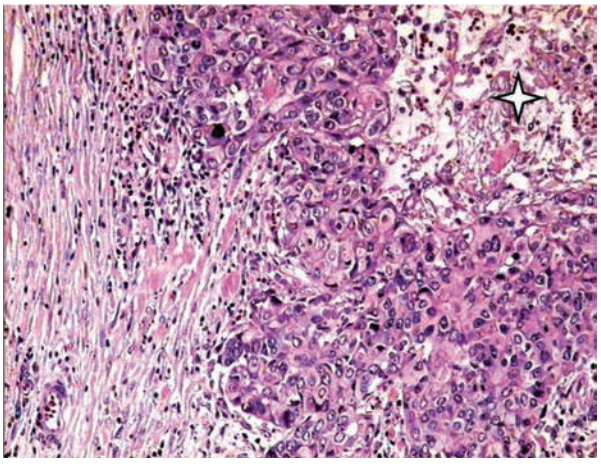

Fig. 1. High grade breast cancer. Note the solid architecture lacking tubular structures, presence of nuclear pleomorphism and mitotic activity. An area of necrosis is also present (marked by asterisk). Haematoxylin-eosin, original magnification 100x.

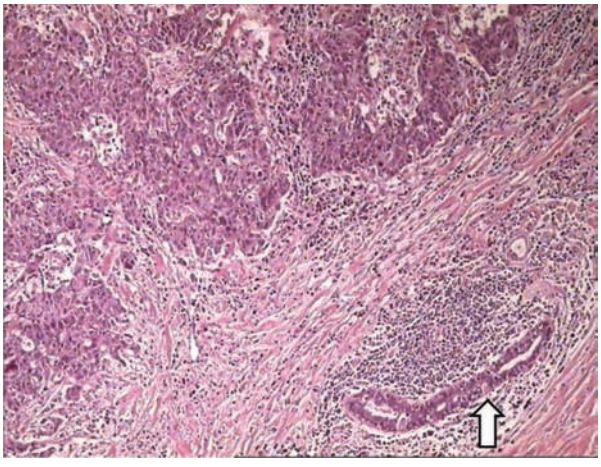

Fig. 2. Invasive breast cancer exhibiting pushing border. Note the benign mammary tissue (marked by an arrow). Haematoxylin-eosin, original magnification 100x.

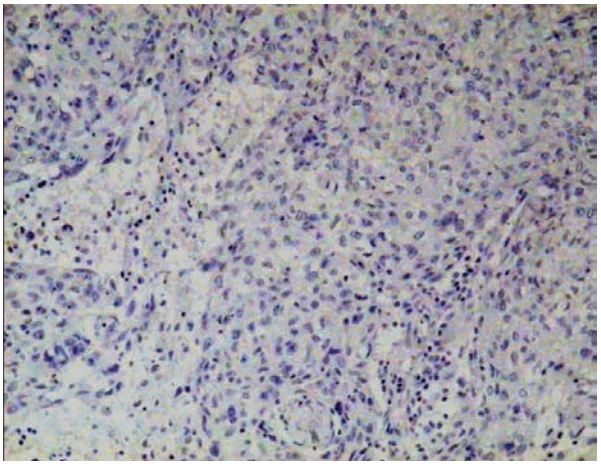

Fig. 3. Lack of estrogen receptor expression in high grade breast cancer. Immunoperoxidase, anti-oestrogen receptor alpha, original magnification 100x.

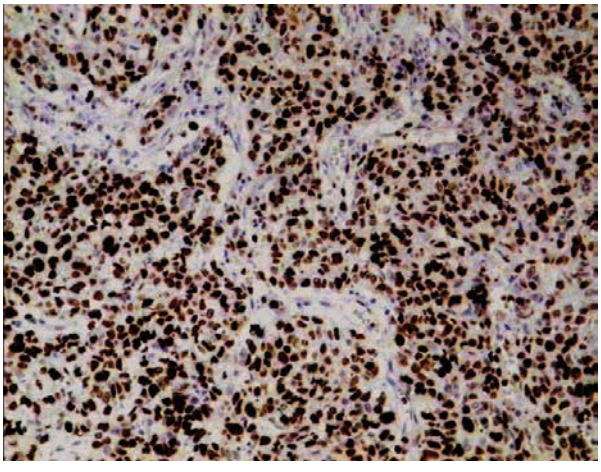

Fig. 4. High proliferative activity in breast cancer by Ki-67 expression. Immunoperoxidase, anti-Ki-67, original magnification 100x.

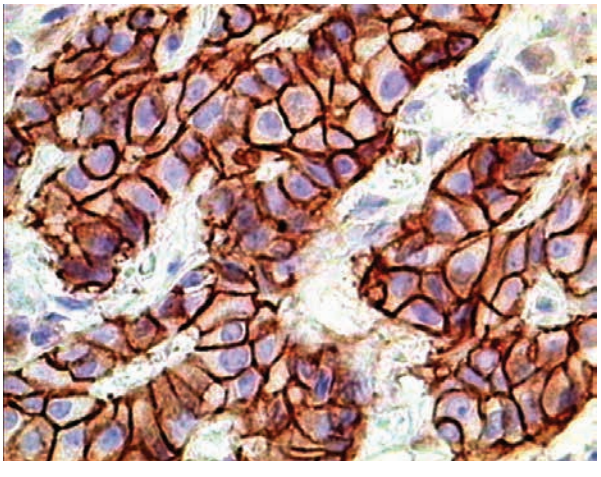

Fig. 5. Intense membranous expression of E-cadherin in ductal breast cancer. Immunoperoxidase, anti-E-cadherin, original magnification 400x.

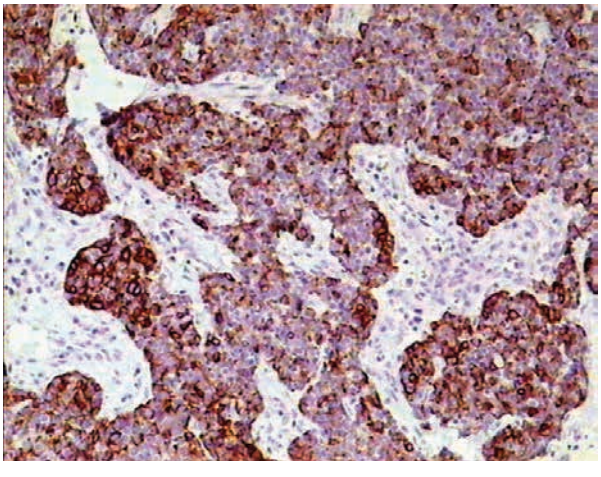

Fig. 6. Heterogeneous cytoplasmic expression of cytokeratin 5/6 in medullary breast cancer. Immunoperoxidase, anti-cytokeratin 5/6, original magnification 50x.

PROBLEM-SOLVING ARTICLE

Prognostic and Predictive Significance of Breast Cancer Stem Cells

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Summary
The existence of tumorigenic (stem) cells has been first described in acute myeloid leukemia (AML), and afterwards in some solid tumors. Recently, they were identified also in breast cancer showing high levels of CD44 surface marker and low level or lacking of CD24. Latest development revealed also significance of presence of ALDH1+ in this certain subpopulation of cells. These cells – CD44+/CD24- with ALDH1 have additional capability to induce tumors in NOD/SCID mice, have been extensively investigated in human tumors, and are claimed by some investigators to be responsible for cancer growth, relapse, resistance to chemotherapy, endocrine and radiation treatment, as well as widespread metastasis in breast cancer patients. However, the hope to solve the problem of breast cancer treatment focusing all attention to breast cancer stem cells (BCSC) has not yet been entirely successful and rather speculative. Investigations in animal models and human breast cancer cells grown as single cell suspensions or mammospheres *in vitro* have enlighted some of the latter problems, but the results until now are scarce and contradictory. In our review we have focused only on problems of vital interest for clinical practice, trying to elucidate the significance of breast cancer stem cells (or broadly speaking tumorigenic breast cancer cells) for prognostic and predictive significance in breast cancer treatment and prognosis. As a rule, BCSC are immunohistochemically detectable, having CD44+/CD24-/^{low} or ALDH1+ as a surface markers, they as a rule present a subpopulation of cells with intrinsic resistance to chemotherapy, some of them (CD44+/CD24$^{-/low}$) to radiotherapy; as to endocrine treatment, no satisfactory data are available until now. The BCSC problem remains unsolved, *in vitro*, *in vivo* or using them as indicators for treatment strategy. More data are to be needed for definite answers to many questions put forward by investigators and clinicians.
Key words: breast cancer, stem cells, prognostic and predictive significance.

INTRODUCTION

Recent studies have identified a small fraction of tumor primary cells with early progenitor phenotypes and genetic fingerprints as the tumor initiators (26, 31). After the publication of Al-Hajj M., et al. 2003, concerning identification of breast cancer stem cells (BCSC) (2)], the stem cell research in solid tumors came once again into the limelight of cancer research, (although the idea goes back as far as 19th century), and human breast cancer gave supporting data for it as a stem cell based disease (4; 13). The interest in stem cells (or tumor initiating cells) reflects not only pure theoretical interest, but mainly clinical, giving additional hope for cancer treatment, especially breast cancer chemotherapy, as well as pour some light on questions, such as metastasis (11; 12; 28; 29). Understanding cancer stem cell biology may lead to insights into the causes and treatment of tumor metastases (9; 22; 23).

A consensus panel of American Association of Cancer Research has defined cancer stem cells as "*a cell within tumor that possesses the capacity to self-renew and to cause the heterogeneous lineages of cancer cells that comprise the tumor*" (7).

Cancer stem cells (CSC) have properties in common with normal somatic stem cells, such as ability to self renewal; differentiation, expression of specific markers and genes, and usage of the same signaling pathways.

At the same time, differences among normal and CSC lies in ability of the latter to metastastatic ability, tumorigenic activities and resistance to chemotherapy (24).

Breast cancer stem cells are thought to be identifiable possessing markers CD44+/CD24$^{-/low}$. However, their numbers vary significantly among tumors, they are not regarded as specific for BCSC, rather for certain histological type (4). They are characterized by CD44+/CD24$^{-/low}$/ESA+ markers, and lineage (lack of expression CD2, CD3, CD10, CD16, CD18, CD31, CD64 and CD 140b). As few as 200 of these subpopulation of cells are able to form tumors injected into NOD/SCID mice, and CD44+/CD24- phenotype has been used extensively to identify cancer cells known for they capability to give rise to tumors in mice. Breast tumor cells positive for ALDH are able to generate tumors with phenotype resembling parental one, therefore CD44+/CD24-/ALDH+ phenotype increases even more the tumorigenicity of breast cancer cells, in comparison with CD44+/CD24- or ALDH+ alone (10).

As far as experimental models are clarified, it seems not to be so clear-cut in human breast cancers (27). Nevertheless, BCSC seems to be intriguing topic for prognostic and predictive purposes in breast cancer.

Although evidence is scarce, it seems to be useful to compile the existing data, especially during last 4-5 years, especially when the stem cell model has not been adequately tested in most cancers (5).

Quantity of distribution of CD44+/CD24$^{-/low}$ and the correlation of phenotype with malignancy grade, estrogen (ER) and progesterone receptors (PgR), HER2 (human epidermal growth factor receptor) etc.

Investigation of expression of CD44+/CD24$^{-/low}$ cells were studied in 60 cases of invasive breast carcinoma no correlation were found, number of cells varied, reaching average 65%, with no correlation with age of patients, presence of lymph node metastasis, tumor size, molecular subtypes and expression of various breast cancer markers in breast carcinoma. No clinical relevance was detected (14). A meta analysis of 12 studies, comprising 898 cases and 1,853 controls have shown, that BCSC positive tumors , particularly those who presented ALDH1 positivity were associated with high histological grade, ER and PgR negativity and HER2 positivity. The investigations revealed poor survival in patients whose tumors presented CD44+/CD24- and ALDH1 cell positive tumors ($p<0.001$) (18). Recently, HER2–mediated tumorigenesis were thought be due to the protein's action on tumor initiating cells or cancer stem cells in HER2 positive cancers, closely correlating with trastuzumab or lapatinib preferential sensitivity and clinical efficacy, witch mainly might be dependent on action on tumor-initiating cells (21). As to Brca1 breast tumors in mice, it is shown, that they contain distinct population of CD44+/CD24- and CD133 cells with cancer stem cell properties, at the same time Brca1 deficient mouse mammary tumors harbors heterogeneous cancer stem cell populations (30).

BCSC and conservative treatment.

Despite recent therapeutic developments, including HER-2 specific agent – trastuzumab, locoregional and systemic disease recurrence remain a threat to higher survival of breast cancer patients. The origin of disease may depend on survived cancer cells, resistant to treatment, possibly, they might present the BCSC phenotype. Several approaches, including specific targeting with drugs, solving the problems of radioresistance and endocrine treatment might include the methods for solving the problem (1, 20).

One of the aims for BCSC detection in tumor specimens is their putative significance in susceptibility to anticancer drugs. However, until now the main investigations are done using single cell cultures and mammospheres. BCSC can be isolated and propagated *in vitro*. So the core biopsies have been done and CD44+/CD24- cells grown *in vitro* as single cell suspensions for testing a new HER2 pathway inhibitor – lapatinib, in HER2 positive tumors. As a result, lapatinib did not lead to decrease of CD44+/CD24$^{-/low}$ cells, at 6 weeks, in comparison with baseline. The study provide a clinical evidence of a subpopulation of chemotherapy resistant breast cancer initiating cells.(12) Also testing the level of expression of ALDH1, CD44+/CD24- cells in 192 human breast tumor samples *in vitro* before adjuvant chemotherapy have shown that the clinical response (PR and CR) was only 52,62% in patients with high ALDH1 expression, while in patients with low expression it was 81,17%, therefore investigators conclude, that tumor initiating cells contributes to chemotherapeutic resistance of breast cancer [8]. Breast cancer samples before primary systemic chemotherapy were obtained from 92 patients receiving either AD (50 pts) or AC (doxorubicin/cyclophosphsmide) - 42 pts. The CD44+/CD24$^{-/low}$ or ALDH1+ phenotypes were estimated immunohistochemically. A higher proportion of these cells were correlated with higher histological grade, ER negativity and high Ki-67 profile in pretreatment samples. On the other hand, after the chemotherapy, ALDH1+ was associated with higher rate of pathologic complete response (pCR) following chemotherapy. In overall, proportion of cells having CD44+/CD24- phenotype increased after chemotherapy. The study provides clinical evidence that putative BCSC are chemoresistant and are associated with tumor progression, emphasizing the need for targeting these cells with anticancer agents (10). Another study concerning BCSC in 108 patients, who were treated with neoadjuvant chemotherapy, consisting of sequential paclitaxel and epirubicin based chemotherapy. BCSC were identified using by immunohistochemical staining of CD44+/CD24- and ALDH1+ in tumor tissue obtained before and after neoadjuvant chemotherapy. ALDH1 positive tumors were associated with low pathological CR (pCR) rate – 9.5%, versus 32.2%, but investigators did not observe significant association between CD44+/CD24- and pCR rates. ALDH1 positive cells significantly increased before and after neoadjuvant chemotherapy in 78 patients without pCR, but the CD44+/CD24- did not. Therefore authors suggest, that ALDH1+ cells play significant role in resistance to chemotherapy (16). Another development seems to be immunotherapy targeting of tumor-associated antigens of BCSC, an approach that might lead to meaningful clinical remissions (17). There is evidence from number of studies, that BCSC are ER alpha negative and EGFR/HER2 positive, which might reflect the resistance of breast cancer to endocrine therapy (18).

Radiation therapy is one of the components of combination treatment of breast cancer patients. Radiation treatment given after surgery in early stage breast cancer patients has been shown to increase the both local control and survival. Post mastectomy irradiation in locally advanced breast cancer similarly improves local control and survival beyond chemotherapy and endocrine therapy. Also the radioresistant phenotype has been hypothesized to reside in the cancer stem cell component. However, the authors found CD44+/CD24- ALDH1 population was rapidly depleted, suggesting sensitivity to radiotherapy, which was followed by decrease in functional BCSC activity , measured by tumor sphere frequency and the ability to form tumors in mice. In contrast, another patient tumor sample they find enrichment of BCSC after irradiation, signifying radioresistance (19). The

same phenomenon of radioresistance was observed also by other authors, namely, the inherited resistance to radiation. It is known, that the success or failure of standard clinical radiation treatment is determined by repair of DNA damage, redistribution of cells in the cell cycle, repopulation and reoxygenation of hypoxic tumor areas. In majority of tumors, this is also attributed to resistant subpopulation of CSC (15). As a method allowing to selectively sensitizing the radioresistant subpopulation of BCSC in order to enhance their response to radiation therapy is hyperthermia. The mechanisms for these effects of hyperthermia are currently under investigation (3).

CONCLUSIONS

Cancer stem cells have been identified in AML, and afterwards in some of solid tumors. As to their role there exists different opinions – meaning their role in formation of metastasis, in long-term dormant state, which gives rise to tumor after many years of eradication of primary tumor, resistance to conservative treatment. However, their predictive and prognostic role has come into the attention for investigators, seeking for explanation of various cases for poor prognosis and survival and resistance to treatment with drugs, endocrine treatment and radiation therapy.

Breast cancer stem cells, are being last years development giving insight into successes and failures of treatment for this kind of cancers. It seems to us, that investigators and doctors should add to their surface marker kit also CD44; CD24 and ALDH1 as most common tools for identifying breast cancer stem cells. If not to-day, in future it will bring benefit to the patients, for treatment choice and explanation of treatment failures in many cases. The investigation in BCSC will go on.

Conflict of interest: None

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PROBLEM-SOLVING ARTICLE

Chronic Pancreatitis: Problems of Classification

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Summary
The article encompasses a review of those classifications of chronic pancreatitis (CP) that are of value for clinical practice. None of them gives a reliable description of the severity of clinical presentation and severity of CP course. Clinical pancreatic index (CPI), introduced by the authors, is a clinical classification aimed at solving this problem. CPI gives a quantitative degree of clinical symptom intensity and the severity of clinical course of CP. The application of CPI is useful both when completing clinical researches and in clinical practice. CPI is designed to help choose the best CP treatment option and its intensity.
Key words: classification of chronic pancreatitis, surgical treatment of chronic pancreatitis, clinical pancreatic index.

INTRODUCTION

Chronic pancreatitis (CP) is continuing inflammatory disease of the pancreas characterized by irreversible morphological changes with abdominal pain and/or permanent impairment of pancreas function (22). Incidence of CP ranges from 1.6 to 23 cases per year per 100,000 people (9). According to the data of autopsy studies, the prevalence of CP ranges from 0.04% to 5% (14, 17). Most patients suffer from debilitating pain which is difficult to manage. Initial conservative chronic pancreatitis treatment often appears to be ineffective, about 30-50% of patients become opioid-dependent (12). Over time, approximately half of patients with CP indication appears for surgery (7). Indeed the idea that surgical treatment is to be applied in chronic pancreatitis sooner and more frequent is widely supported. Such approach could improve quality of life of the patients as well as reduce treatment costs. Surgical treatment usually is applied in case of resistant chronic abdominal pain, in case of complications, in suspected neoplasia. According to the reported data, surgical treatment is effective in case of recurrent acute exacerbations of CP (13). The next surgery types are applied to reduce pain in CP: resection (pancreatic head resection, distal pancreatectomy, total pancreatectomy) and decompression (lateral pancreaticojejunostomy, cyst-enterostomy, sphincterotomy). Alternative approach – nerve ablation procedures – has no prolonged influence on pain. The choice of the surgery depends mostly on the pain intensity, pancreas ductal anatomy, presence of exocrine/endocrine insufficiency, presence of complications. Such surgeries reduce pain for a long time in 60-80% of the patients (21). However, at present surgery is not a first-line treatment. For timely and reasonable treatment choice, including surgery, uniform recommendations are necessary. Valid clinical classification could help in such recommendations creation.

Despite the fact that during the last years more than 10 types of chronic pancreatitis (CP) classifications were presented, problems with disease classification still remain. CP can have different types of etiology, pathogenesis, clinical course and many complications. The first classifications are limited as describe just certain field. Thus, Marseille classification (1963) considers hydrological pattern of the CP, Marseille-Rome classification (1988) includes some etiology and pathogenesis of the CP, Cambridge classification (1983) describes radiology data only (18), TIGAR-O classification (1994) mentions just probable risk factors (5). The Zurich classification (1) contains CP diagnosis criteria, describes etiologic factors and stages of illness. Even though diagnosis criteria in this classification are elaborated for alcoholic pancreatitis, they can be used to verify non-alcoholic CP etiology (1). Such classification distinguishes only two stages of disease – early stage and late stage, giving a very scarce clinical presentation of disease, which limits its application in clinical practice. Japan Pancreas Society classification (10) describes CP diagnosis criteria basing on USG data, ERCP data, secretin test and pancreas histology. This classification also does not consider the clinical presentation and the clinical course, being quite limitedly used in clinical practice.

Since year 2000, new CP classifications more or less describe clinical presentation and the severity of clinical course. There are four such classifications currently, each having certain advantages and disadvantages. In 2002, Ramesh offered ABC grading system of CP, which is a clinical classification, distinguishing types of disease into three groups, depending on complications and the presence of pain (16). Stage A is defined for patients with CP without pain, stage B – in case pain is present and there are no complications, stage C – if pain and complications are present. Besides that, it is offered to indicate endocrine and exocrine impairment. In addition to defining the stage of illness according to ABC grading system, Ramesh advised to indicate its etiology. In accordance with this grading system, stage A requires treatment of diabetes and steatorrhea only, stage B, as well as medical therapy, may need endotherapy and surgery, while stage C mainly requires endotherapy

and surgery. The advantage of the given classification is an opportunity to categorize patient more precisely, which promotes the right choice of treatment (4). The disadvantages of such classification include the inability to distinguish severity of disease inside every group and the absence of all possible clinical presentations of the disease (19). Besides that, the given classification was tested in a research involving young men with tropical pancreatitis, so more researches are needed to prove the relevance of such classification of CP for cases of different etiology. Some authors assume that this classification is having excessive parameters (6).

Manchester classification system for CP (3) was developed in 2006. Its authors presented an idea of distinguishing the stages of CP (mild, moderate, end-stage) in order to apply them in clinical practice. The criteria of defining stages were basing ERPC/CT data, the presence of abdominal pain, the use of opiates, the assessment of exocrine and endocrine function, the presence of peri-pancreatic complications. Even though the classification system for CP is quite simple and easy to use, it was criticized by several authors who consider such staging to be imprecise and not covering all possible clinical presentations of CP (19).

In 2009, Büchler in association with co-authors offered another type of CP clinical classification, which supposes distinguishing three stages (A, B and C) basing on clinical symptoms of disease and the data of imaging technique (6). The basic idea of such classification is to consider disease etiology, at least one clinical criterion, as well as the data of visualisation techniques or functional tests, which approve changes relevant to CP. It is advised to consider pain, attacks of acute pancreatitis, complications of CP, steatorrhea and diabetes mellitus among clinical criteria. Stage A is early stage, B – intermediate stage, C – end stage of the disease. For stage A pain of any type and degree and/or attacks of acute pancreatitis without complications, without steatorrhea and insulin-dependent diabetes mellitus, is characteristic. Stage B is described with complications, but excluding steatorrhea and diabetes mellitus. The key feature of stage C is exocrine and/or endocrine function loss and along with this, CP complications can both be present of absent. Such classification is quite easy to use as being fairly laconic. In our opinion, the given classification is logic from the point of view of disease stage description according to chronology and main clinical problems, specific for every stage. The given classification works effectively for prognosis of the future disease course. As to the application of this classification of CP for planning the treatment mode, it must be admitted that its opportunities are limited due to a scarce description of clinical aspects.

The approach of distinguishing stages of disease is quite popular in medicine. CP staging must help in prognosticating disease course (3) and choosing the right treatment mode (2). Nevertheless, the staging of CP is relevant to patient treatment in general terms only. The choice of certain CP treatment mode (conservative, endoscopic and surgical) and its intensity is still made individually and depending on many factors – etiology, clinical hints, type of complications and the presence exocrine/endocrine insufficiency. During the last years, the opinion that the choice of CP treatment mode largely depends on clinical symptoms and findings and their combination remains unchanged. In case of diseases with exacerbations and remissions, there is a need to describe the "expansion" of disease and its activity separately. All classifications described above contain quite extensive information about possible clinical findings, types of morphological and functional abnormalities, as well as CP developmental stages. This information is very important, but, unfortunately, is of „extensive" character, giving a very general description of disease symptoms in relation to patient and chronology. When curing CP, it is vital to have the notion of disease activity in every period of time, considering the seriousness of clinical aspects. This bespeaks the choice of the best treatment and its intensity.

In 2007, Schneider with co-authors presented a very detailed M-ANNHEIM classification of CP (19), which is considered to be the most full current version of classification. Its authors completed a huge work and studied the data of many epidemiological, clinical, genetic and experimental researches devoted to CP. The given classification contains the definition of disease and its complications, possible risk factors and diagnostic disease criteria. Cambridge classification is used as imaging criteria of CP (18). Besides that, the classification puts emphasis on everything dealing with the clinical presentation of CP, especially the staging of disease with the possible clinical presentation of the disease on every stage. Unlike the other CP classifications presented before, M-ANNHEIM classification of CP contains scoring system to determine the severity of the disease. The severity index of CP involves clinical features of CP, such patient report of character of pain (no pain, freedom of pain between attack of acute pancreatitis, no pain with therapy, intermittent pain, continuous pain), pain control with medication, pancreatic surgical intervention, occurrence of endocrine and exocrine insufficiency, morphologic status on pancreatic imaging, occurrence of severe organ complications (e.g. ascite, bleeding, pseudoaneurism etc.). Basing on summarized severity index of CP, minor, increased, advanced, marked and exacerbated severity levels of the disease are distinguished. This classification was used in clinical research (11), which proved that it is able to evaluate the clinical and severity stages of CP. In another research related to the SPINK1 N34S mutation in patients with alcoholic chronic pancreatitis clinical analysis was successfully approved using the criteria of the M-ANNHEIM classification (8). It should be mentioned that both of these researches had no relation to the evaluation of CP treatment efficiency.

The further perfection of CP clinical classification must be aimed at a more precise definition of disease clinical course severity. Unfortunately, the abovementioned clinical classifications do not mention all significant clinical symptoms when describing stage criteria and CP

severity. None of the described CP classifications does contain sufficient criteria to define the degree of clinical symptoms such as pain, body weight loss, defecation frequency, steatorrhea degree, etc. The perfect tools for assessing CP clinical course severity are the criteria which give a quantitative or a semi-quantitative description of all significant clinical signs of the disease.

In 2007 we developed a CP clinical course severity classification (15), named Clinical pancreatic index – CPI. CPI is based on formalization of such criteria of disease severity as number of operations related to CP, number of hospital courses related to CP exacerbation, the degree of weight loss, pain intensity, steatorrhea degree, number of defecations per 24-hour period, glucose tolerance impairment (Table 1).

Table 1. Qualitative assessment of CP clinical course using clinical pancreatic index.

Sign		Assessment, score
Number of surgical operations related to CP complications	0	0
	1	1
	2	2
	3 and more	3
Efficiency of out-patient treatment	efficient	0
	non-efficient	2
Number of hospital treatment courses with CP in anamnesis	0	0
	1	1
	2	2
	3 and more	3
Body weight loss (kg)	0	0
	1,5	1
	3 - 5	2
	more than 5	3
Pain intensity (partly according to visual analogue scale)	no pain	0
	mild pain	1
	moderate pain	2
	severe pain	3
Steatorrhea degree (semi-quantitative method)	0 or +	0
	++	1
	+++	2
	++++ and more	3
Average number of defecation per 24-hour period	0	0
	1	1
	2	2
	3 and more	3
Another dyspeptic complaints (abdominal bloating, borborygmus, nausea, postprandial discomfort)	1	0
	2	1
	3	2
	more than 3	3
Glucose tolerance	not impaired	0
	impaired	1
	diabetes mellitus	2

The intensity of pain is assessed according to visual analogue scale, where 1-3 points correspond to mild, 4-7 points – to moderate and 8-10 points – to a severe pain. A higher score of CPI assessment corresponds to a more severe course of CP. The maximum CPI is 25 points. Depending on total score, mild course (6-8 points), moderate severity course (9-15 points) and severe course (16-25 points) are distinguished when describing CP. CPI is a simple and easy practical classification of CP course severity. It does not need any invasive procedures, complicated or unique researches, so it can be applied in all clinics. In the clinical study (20) we completed in 2009 the objectivity of CPI was proved. This study results showed the correlation between CPI and quality of life data (using EORTC QLQ-C30) for patients with CP both before and after the course of conservative therapy. This gives a reason to suppose that CPI can be widely used in clinics for many purposes. Firstly, CPI indexes of patients with CP aggravation can be used when conducting clinical researches aimed at defining the efficiency of any CP treatment mode applied – conservative, endoscopic or surgical. This gives a chance to elaborate unified recommendations for CP treatment depending on disease course severity. Secondly, CPI gives an opportunity to monitor clinical aspects of CP in any period of time. CPI also helps to approve CP aggravation and choose the proper way and intensity of treatment. Thirdly, CPI aids in assessing the effectiveness of the applied treatment for a certain patient, and, if it turned out to be inefficient, helps to choose a successful alternative treatment. We suppose that such classification of CP course severity is applicable to all etiologies and stages of CP, because it is based on clinical signs of disease.

CONCLUSIONS

Over the history of CP researches a lot of disease classifications were elaborated. Only a part of these are clinical classifications. For a long time it was a challenge to develop a clinical classification that would give a reliable description of clinical presentation severity and CP course severity. Such classification is needed for a more precise division of patients into groups when completing clinical researches and comparing their results. Given the wide range of CP treatment options,

such accurate clinical classification of the disease, that takes into account the severity of the clinical course of disease, is required. We elaborated CPI, which allows to get a quantitative assessment of clinical symptom and disease course severity. Future researches are needed to define opportunities of CPI application both in clinical practice and when making clinical researches, which is the further goal of the authors.

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PROBLEM-SOLVING ARTICLE

Potential Role of Cytokines in Children with Acute Appendicitis and Acute Mesenteric Lymphadenitis

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Summary
Although, AAP and AML have different etiological factors, clinical symptoms are very much similar but treatment tactics in both the disease differ a lot. In case of AML, treatment is more conservative and does not require hospitalization while in case of AAP immediate hospitalization and maybe further surgery can be mandatory. With the identification of group of cytokines serum inflammatory mediators IL-8, IL-10, IL-12[p70], IL-17, TNF-a and MCP-1, it is believed early and proper diagnosis of AAP in the near future. Research of cytokines-serum inflammatory mediators has opened new opportunities for an early detection and differentiation of these two diseases in children.
Key words: cytokines, serum inflammatory mediators, acute appendicitis, acute mesenteric lymphadenitis.
Abbreviations: AAP - acute appendicitis; AML - acute mesenteric lymphadenitis; CT - Computer tomography; US – Ultrasound; CGSIM - group of cytokines in serum inflammatory mediators; TNF - tumor necrosis factor; CSF - colony stimulating factor; CRP - C- reactive protein; SAA - serum amylase A protein; MBP - mannose binding protein; NK - natural killer cells; MCP-1 - monocyte chemo attractant protein; Th1 - type 1 T-helper cells; Tc - cytotoxic T lymphocites.

INTRODUCTION

In children the most common and important surgical diagnostic problem in emergency as well as outpatient department is the abdominal inflammatory process i.e. sudden onset on abdominal pain. Around the world including in Latvia, acute appendicitis (AAP) and acute mesenteric lymphadenitis (AML) are dominating inflammatory processes in children requiring urgent diagnosis along with proper treatment. Each year in U.S. in pediatric units 77000 cases are treated with the suspicion of AAP, while in U.K. 44000 patients are admitted each year with acute abdominal pain with the suspicion of AAP (3,8). In Latvia itself each year around 3000 children are sent to the pediatric surgeon with acute abdominal pain, 500-600 of these patients undergo surgical treatment in first step but in 35-37% of cases the diagnosis is delayed and treatment is prolonged due to complications of the underlying disease. In order to reduce the complications it is really important early and accurate diagnosis of AAP in children (3). Overall, the incidence of AAP is directly proportional to the complications associated with its late diagnosis. In children of age group of 10-17 years, AAP with perforation occurs in 18-20% of cases, secondary wound infection in the post operative period in 0-11% and pelvic abscess in 1.5-5% of cases (3,18). All the above mentioned conditions add on to extend the hospital stay, return to normal lifestyle and increase the risk of development of gastrointestinal impenetrability due to the history of complicated intra-abdominal infection (18). Early differentiation of AAP from other abdominal inflammatory pathologies is difficult. Diagnosis in children under difficult objective circumstances also limits the accuracy of assessment of disease especially in case of physically disabled child. AAP presents with different clinical manifestations in different age groups in children, while mesenteric lymph node hyperplasia is quite common in teenagers (18). Although in the last decade there has been a significant progress in the differential diagnosis of AAP from AML, with a significant improvement in diagnosis of AAP from 58% to 71% with the use of Ultrasound (US) and Computer tomography (CT). However, the risk of perforation and negative appendectomy still remains high (6,18). The use of CT is limited in case of children due to the increase in radiation risks (6).

By reducing the rate of negative appendectomies in children, it is possible to reduce the risk of cancer later in the life. The study carried out in Sweden shows increased risk of gastric cancer and non-Hodgkin's lymphoma in patients, 15 years later in their life, who underwent appendectomy in childhood between 10-19 years of age (4). AML is considered really important in the differential diagnosis of AAP in children with the increasing use of US and CT. In the literature, AAP and AML, specific, early differential diagnostic criteria are discussed either too little or in some there is no mention at all. Research of cytokines-serum inflammatory mediators has opened new opportunities for an early detection and differentiation of these two

diseases in children. Currently, AML is diagnosed in patients on the basis of clinical data evaluation and US - increased size of mesenteric lymph nodes more than 10 mm (19). Despite the fact that US has high sensitivity (86.7%) and specificity (90%) in diagnosis of AAP, false negative results of US cannot be ruled out. Therefore US is considered as just one of the diagnostic methods (25). Although, AAP and AML have different etiological factors, clinical symptoms are very much similar but treatment tactics in both the disease differ a lot. In case of AML, treatment is more conservative and does not require hospitalization while in case of AAP immediate hospitalization and maybe further surgery can be mandatory. With the identification of group of cytokines serum inflammatory mediators IL-8, IL-10, IL-12p70, IL-17, TNF-a, MCP-1, it is believed early and proper diagnosis of AAP in the near future.

Specific cytokine pattern

Worldwide over the past decade there has been considerable research in this field of group of cytokines in serum inflammatory mediators (CGSIM) to demonstrate the specificity and early diagnosis of AAP in children. CGSIM are a biological active substance of polypeptides and glycoproteins with a molecular weight of 8-30 kd, which participate in the cellular immunity in response to specific inflammatory process in the body (5). The formation of lymphokines, monokines, tumor necrosis factor (TNF), colony stimulating factor (CSF) and various growth factors are considered to be CGSIM (20). When analyzing various studies, the diagnostic role of CGSIM is not emphasized in cases of AML. It is possible that these changes are not stressed in CGSIM studies when AML subgroups were analyzed and described as negative appendectomy results in cases where appendix was histological unchanged. Since the early diagnosis of AAP in children aged between 1-19 years is closely related to the leukocyte count and the differential leukocyte count changes in the blood, most authors in their publications try to compare this independent inflammatory changes in the blood of active phase protein or C-reactive protein (CRP) and other specific serum inflammatory mediators (2,6,7,9,21,24). Based on these studies, white blood cell count ≥10x10(6)/L and CRP≥8 mg/L in the blood is an essential laboratory AAP diagnostic criteria (2). One of the most frequently mentioned in the literature and analyzed CGSIM is IL - 6 (6,7,10,21). IL-6 diagnostic significance is explained by the increased excretion of macrophage cells, localized and concentrated in nodules near to the in point of inflammation. As a result, IL-6 early effects on hepatocytes and stimulates the acute-phase proteins: CRP, fibrinogen, serum amylase A protein (SAA) and mannose binding protein (MBP) (7,19). IL-6 in the blood in case of AAP is still being debated. Some authors argue that IL-6 specifically increases in the serum only in the case of a complicated AAP (21). Others think that IL-6 also increases in the serum in cases of non-complicated AAP. The literature results, firstly, may have differed due to the inclusion of a population of patients in each study. The studies where IL-6 levels were elevated in children with complicated AAP the diagnosis of AAP in that population was confirmed by histopathology and the exact timing of cytokine assessments were not fixed (21). The other studies where IL-6 levels were found significantly increased in the cases of non-complicated AAP the exact time of sample collection was fixed but the inclusion of a population of patients mostly did not have AAP (10). IL-6 diagnostic accuracy is totally dependent on the CGSIM sample collection time during the phase of AAP because IL-6 concentration is up-regulated by mRNA transcription in the macrophage cells and its maximum concentration is reached after 4-6 hours from the onset of disease (14).

Talking about diagnosis of AAP, the next most studied so far CGSIM is IL-8, which increases in case of complicated or perforated AAP (1,5,10,11,14). Increase of IL-8 is observed in patients with complicated AAP after 24 hours of onset of symptoms (10). In this case CGSIM releases activated monocytes and macrophages. IL-8 is considered highly selective cell binding factor which helps in attaining high concentration of neutrophil cells in the inflammatory tissues and its cell lysis, promoting deeper inflammatory response (5). Proving the expression of the gene (IL-8 mRNA) for neutrophil chemoattractant IL-8, isolating it from pathologically altered appendix tissue, indicates new directions in pathogenesis of AAP and also different immune response pathways in the appendix in case of inflammatory process. Therefore, IL - 8 concentrations in serum and exudates from the abdominal cavity are significantly correlated with the histological changes in the AAP (5,12).

So far in the literature of CGSIM, less analyzed is IL-10, secreted by activated macrophages and T- helper cells (Th) and its main function is to ensure homeostasis and inhibition of activated macrophages in order to achieve balance in cellular immune responses (5). IL-10 level in the serum is increased in cases of gangrenous AAP as a cellular immune response to type 1 T-helper cells (Th1) activity, in return stimulating the cytotoxic effect of the appendix and its perforation. Cytotoxicity is characterized by a single cell ability to kill other. This main function is performed by cytotoxic T lymphocytes (Tc) or CD8+ cells which convey the apoptosis signal to destroy marked cell. Increased IL-10 levels are not only observed in the serum but also in the exudates from the abdominal cavity in case of AAP (16,26).

While analyzing the literature available regarding IL-17 as one of the diagnostic criteria in diagnosis of AAP in children, we observed that IL-17 changes in the serum are still very little explored. A Swedish study demonstrated that IL-17 increases in the serum in case of gangrenous AAP. It makes us think that phlegmonous and gangrenous appendicitis have different immune response reaction to the inflammatory process of the appendix (17). Cells that produce IL-17 are T lymphocytes (CD4+ cells) by the stimulation of IL-6, IL-8, IL-1, EPO secretion from epithelial, endothelial and fibroblast cells (9). IL-17 is essential in the pathogenesis of AAP as it stimulates the proliferation of fibroblasts

in the presence of CD34+ cell and the maturation of neutrophils (9).

Quite a little is mentioned about IL-12[p70] in the literature. This CGSIM stimulates mature T lymphocytes (CD4+ cells) and natural killer cells (NK). Experimentally, as IL-12 enters the organism, it increases extramedullary haematopoiesis in the spleen with bone marrow hypoplasia and pancytopenia (20). In cases of AAP and histopathology unchanged appendix, IL-12 changes in the serum concentration is considered as unsufficent (15). It is further required some specific studies to accept or deny this hypothesis.

The CGSIM subgroup of TNF-α is released from the macrophages, monocytes, lymphocytes, keratinocytes and fibroblasts in response to the inflammatory process. Increase in its concentration is observed in case of uncomplicated or phelgmonous AAP (13,20). However, there are conflicting studies, which reflect a slight correlation between origin of appendicular inflammation and TNF-α concentrations in serum (21,23).

One of the least analyzed CGSIM is MCP-1 (Monocyte chemoattractant protein) which activates and attracts monocytes and macrophages to the inflammed tissue (20). *In vivo* high concentrations of MCP-1 stimulate macrophages and monocytes activity. Significant MCP-1 changes were observed in children after laparoscopic apendectomy performed due to the suspicion of AAP without perforation (22). MCP-1 could possibly be specific CGSIM for early diagnosis of AML, taking into account the specific activity of monocytes.

CONCLUSIONS

The current available clinical diagnostic methods - CRP, complete blood count and US do increase the diagnostic specificity of AAP but fail to present a clear picture of the inflammatory process in the abdomen. As a result, the precise time and efficacy of treatment is sometimes difficult. The available CGSIM scope of research in pediatric patients is limited and rise a lot of controversies. Further research in the field of CGSIM in children with emergency abdominal pain most likely will improve the diagnostic accuracy of its severity and prognosis as well as help in differentiating AML from AAP.

Conflict of interest: None

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PROBLEM-SOLVING ARTICLE

Invasive and Non-invasive Methods in Diagnostic of Migraine: a Literature Review

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Summary
Migraine is one of the most common neurological disorders worldwide. Prevalence varies in different reports but on average it is up to 15% of adults, mostly women, and up to 14,5 % in school age children. Although the diagnosis of migraine in children is not easy, migraine prevalence of migraine in childhood is very high. Morbidity of migraine is strongly related to positive family history and it illustrates ascertain close relation to genetic matter. Medical and social burden of migraine is tremendous since it affects all range of ages, starting from young children to seniors, and severe attacks can be even as disabling as quadriplegia. Despite there are thousands of trials done, indisputable cause of migraine as well as pathophysiological treatment is not clear. There are several forms of migraine, two of which are the most common- migraine without aura and migraine with aura. Diagnosis of migraine is based mainly on anamnesis data and clinical symptoms according to The International Classification of Headache Disorders (ICHD), neuroimaging is recommended only in case of so called red flag symptoms and is oriented to exclude secondary headache. Invasive investigations such as lumbar puncture and angiography with contrast are integral parts of investigational plan when migraine has to be differentiated from subarachnoid haemorrhage or pulmonary and cardiac right-to-left shunts. Modern neuroimaging reveals migraine as a kind of neurodegenerative disease that in severe and/or frequent form leads to brain white matter lesions, subclinical infarcts in the posterior circulation and increased iron deposition in brain. On the scientific laboratory level, it is possible now to diagnose migraine on affirmative base, and this is believed to be the future of neuroimaging in clinical practice regarding migraine.
Key words: migraine, neuroimaging, lumbar puncture, subarachnoid haemorrhage, iron deposition, white matter lesion.

INTRODUCTION

Despite the fact that migraine is a common neurological paroxysmal disorder, it is still under-diagnosed and under-treated illness (7). From primary recurrent headache suffer patients of all ages, but prevalence reaches apogee at age of forty and then decreases. Interestingly, that migraine in pre-puberty is more common in boys than in girls, but in adolescence and adulthood the prevalence is almost three times higher in women (2). Lewis et al in 2000 found out the prevalence of migraine in school-aged children up to 7%, but in adolescent girls- two to three-fold higher- 14 to 20 %, but several years later Ozge et al reported prevalence of migraine in school age children with much higher percentage- from 3,2 to 14,5%, and drew attention on significant and reliable headache-positive family history (16, 21). Goadsby et al (2006) referencing on World Health Organization stated that migraine has to be considered as serious illness that can be even as disabling as quadriplegia. From migraine suffers about 15% of adult population worldwide. Diagnosis of migraine in children is complicated due to varying migraine symptoms and difficulties for the child to interpret the pain (10). It should be noted that significant part of adult migraine patients who suffers from severe and/or frequent migraine used to overuse medications thus entering kind of *circulus vitiosus*-medication usage because of severe illness causes medication overuse migraine, which often manifests as chronic daily or near-daily, therapy-refractory headache (5). Chronic daily headache (CHD) according to definition is headache persisting at least 15 days per month, for at least 3 consecutive months, for 4 or more hours per day. Due to severity of pain, CDH is the most common reason for consultation in headache clinics (3). Despite the severe clinical manifestation of migraine, some authors have concluded that even up to fifty percent of migraine cases remain undiagnosed due to poor socio-economic and educational status of sufferers, and low disease awareness in society (14).

There is no epidemiologic data available for migraine in Latvia so we should assume the prevalence of disease is close to worldwide statistics.

The hypotheses about the origin of migraine have been considerably changed during last fifty years; nevertheless pathophysiology of migraine is still not fully explained and understood. The most popular opinion is that the pain originates from chemical activation of sensory nerves of intracranial blood vessels and meninges (24). The range of recent reports draws attention on causal relationship between migraine and different right-to-left shunts, cardiac and pulmonary. Pulmonary arteriovenous malformations (PAVMs) with a high prevalence (around 50%) are found in migraine and hereditary hemorrhagic telangiectasia (HHT) patients. Migraine may be the initial and/or the only symptom of PAVMs. The fact that embolization or surgical resection of PAVMs decreases the severity and frequency of migraine attacks indicates the causal relationship

of both diseases (20, 23). Some trials have reported cardiac right-to-left shunt patent foramen ovale (PFO) percutaneous closure as a way for improvement of migraine frequency and severity. Nevertheless there is no convincing scientific evidence to support PFO closure for treatment of migraine (26). Recent achievements in neuroimaging support the hypothesis that migraine as well as other severe chronic pain can induce changes in the brain that sequentially could lead to functional changes. The common conclusive view perceiving migraine as benign disorder which does not induce any pathological changes in brain has been challenged now by a lot of trials reporting detectable changes in the brain in case of severe and/or frequent migraine attacks (6, 28).

Non-invasive and invasive diagnostic methods of migraine

Rapid development in human brain imaging with an aim to understand the link between pain and structural changes in brain started around 20 years ago. Three main directions of methods and techniques have been used to get an „image of pain": 1) structural techniques that supply an information about anatomical status of the brain (such as changes in brain volume): structural magnetic resonance imaging (MRI), diffusion tensor imaging (DTI); 2) chemical or biochemical techniques that give information about biochemical status of the brain: magnetic resonance spectroscopy (MRS); 3) functional investigations, that reveal neuronal activity and illustrate an altered state in the brain: electroencephalogram (EEG), magneto-encephalogram (MEG), functional magnetic resonance imaging (fMRI) and positron emission tomography (PET). All the methods complement each other in obtaining full picture of processes in the brain during migraine attack and interictal phase (4, 13).

DTI is a magnetic resonance imaging tool used to visualize the diffusion of water in the brain. DTI has the possibility to detect direction of water diffusion that is anisotropic in white matter tracts (28). DTI has been used also to discover the effects of aging of human brain as well as for detection of white matter affecting degenerative disorders. Migraine patients with high attack frequency are found to have decreased concentration of white matter in frontal and parietal areas, and this could be evaluated as a sign that frequent migraine attacks lead to white matter threat (30). One of the DTI parameters is functional anisotropy (FA) which reflects cellular and sub-cellular components and allows identifying tracts separately. DaSilva et al revealed that migraine patients were found to have the lowest FA values localized in two regions of the brain- the posterior limb of the internal capsule and the corona radiata. FA in the ventroposterior medial thalamus was different in patients with migraine with aura, migraine without aura and healthy volunteers. In migraine with and without aura the lowest FA were found in different specific regions- in ventral trigeminothalamic tract and ventrolateral periaqueductal grey matter accordingly. The results of trial show that long-term migraine suffering can lead to diffusional changes not only during migraine attack, but also interictally. Periaqueductal grey matter acts as a pain neuromodulator and is critically important for the activation of the nervous system in migraine. In migraine sufferers periaqueductal gray matter contains more iron than healthy controls, and iron level is higher in those with longer duration of migraine (6).

Chemical changes in brain are detectable by using magnetic resonance spectroscopy (MRS). The hypothesis that glutamatergic metabolism would differ in migraine patients and in healthy controls was examined. Clinical data support the notion of migraine induced changes in aminergic function, including alterations in glutamatergic and glutaminergic systems. Interestingly, that difference was detected, comparing not only migraine sufferers with controls, but also comparing such migraine sub-types as chronic and episodic migraine. Glutamate (Glu) and glutamine (Gln) levels in cerebrospinal fluid and plasma were found to be increased only in chronic migraine patients, and higher Glu levels have been reported in migraineurs during the interictal phase, not attack. Since results do not match in different migraine populations reported by different authors, further investigations are needed (24, 28).

Functional brain imaging- positron emission tomography (PET) is useful in detecting activation in the brainstem during migraine attack, which is not present interictally. Another functional investigation- blood oxygen level dependent (BOLD) contrast functional magnetic resonance imaging can be used in studying single patient and detecting the precise site of abnormal activation. In a range of trials magnetic resonance angiography (MRA) was used in migraine patients with the aim of illustrating the cause of headache. Functional MRI has been proven to be useful also in detecting allodynia which is a frequent clinical feature of migraine (18).

In summary, functional imaging is able to detect changes in the dorsolateral pons during migraine attack and findings indicate dorsolateral pons being pivotal in migraine attack (10).

Lewis et al retrospectively analyzed five studies with the question whether neuroimaging is useful in the evaluation of children suffering from headache with no pathological signs in neurological examinations found. Although abnormalities were found in 10,5% children imaged, authors did not suggest to include neuroimaging in recommendations as routine use in paediatric patients over 6 years of age with uncomplicated migraine or chronic daily headache, and normal general and neurological examination due to majority of abnormalities were considered as nonsurgical (16). Only for children with abnormal neurological examinations CT or MRI should be indicated. Two years later Alehan et al examined MRI scans of neurologically normal children with recurrent headache; 22% were abnormal, but only 10% out them was suspect to headache. The authors' conclusion coincided with previous recommendations suggesting neuroimaging only for those recurrent headache

patients who have pathological neurological findings. A change in headache symptoms or personality can be evaluated as a red flag status and neuroimaging may be helpful in differentiating a cause of headache (1). Ozge et al (2011) stated that indications for neuroimaging in patients suffering from headache are focal and/or progressive neurological symptoms. Neurophysiological examinations widely used in last century for diagnostic of headache (electroencephalogram etc.) have a little value in routine headache patient evaluation (21). In diagnosis of pulmonary arteriovenous malformations a high resolution CT scan of the thorax and angiography of the pulmonary vessels are valuable and informative (23). Transthoracic 2-dimansional echocardiography (TTE) with saline contrast injection can be used for detection of patent foramen ovale (PFO) (26).

Neuroimaging and invasive investigation methods in clinical practice

Goadsby P (2004) pointed out the yield of neuroimaging only in differentiating primary from secondary headache when so- called red flags appear. Although the presence of red flags itself does not mean secondary headache, nevertheless when red flags are defined, neuroimaging and other appropriate investigations are indicated (9). Bigal et al have defined that red flags are: sudden- onset headache; worsening-pattern headache; headache with cancer, HIV or other systemic illness (fever, neck stiffness, skin rash); focal neurological signs or symptoms that differ from typical visual or sensory aura; papilloedema; headache triggered by cough, exertion or Valsalva test; headache during pregnancy or post-partum. Lack of response to appropriate treatment can be considered as a red flag too. In all mentioned cases neuroimaging is recommended, as well as lumbar puncture, biopsy, blood test and other appropriate targeted investigations should be considered. Lumbar puncture is useful in determining the presence of infection or blood in cerebral spinal fluid or increased intracranial pressure. Neuroimaging is an essential part of investigation plan for differential diagnostis in patients with daily or near daily headache (3). The major concern for severe migraine patients and often also for doctors is exclusion of life threatening brain diseases, and MRI seems to be an easy way to clarify this. However, brain tumors, for instance, are found in less than 0,1% of patients with the headache history during lifetime. Severity is not a marker of secondary headache since the most severe are exactly primary headaches. Costs for MRI are rather high worldwide and this fact urges healthcare administrators to limit the use of this investigation to minimum, issuing restricting recommendations. On the other hand, reassurance of headache patients and involved medical staff by neuroimaging may lead to cost savings in less calls to emergency departments, neurologists' and GPs' offices later (9, 10).

Very important and challenging is diagnostic of subarachnoid haemorrhage when patient has the history of primary headache and is neurologically intact. Recent recommendations suggest the patients with suspected subarachnoid haemorrhage should be examined by ordinary computed tomography and following lumbar puncture if the scan result is negative. Underestimation and misdiagnosis of subarachnoid haemorrhage can lead to abrupt clinical worsening due to re-bleeding (22).

In emergency department difficulties in migraine diagnosis can occur also in differentiating headache from life not threatening disorders such as subarachnoid haemorrhage. One of the most common is migraine with coexisting sinusitis, when is very important not to dismiss or under-estimate a diagnosis of migraine. The result of CT imaging of the sinuses may emphasize sinusitis, but in the presence of migraine clinical symptoms may not correlate with the scan result. Emergent neuroimaging is recommended only in the presence of red flag symptoms in a patient with so far typical migraine pattern (7).

Helenius et al using conventional and diffusion-weighted MR imaging examined healthy volunteers. Authors' invaluable contribution is defining of absolute apparent diffusion coefficient (ADC) values in different parts of the normal human brain and the results can be used for reference in clinical settings and further studies. ADC values in the normal brain are not significantly affected by aging, have no sex difference and have no difference between the brain hemispheres (11). Rocca et al studied patients with prolonged suffering from migraine (mean disease duration more than 24 years) on DT MRI at high field strength and defined that migraine patients had significantly reduced mean diffusivity histogram peak height of the gray matter, compared with healthy controls. Authors suggested that grey matter damage in migraine patients could be related to cognitive changes (25). Mulder et al already in 1999 raised two questions: whether migraine patients could have cognitive impairment in the interictal period and during post-attack period, compared to matched healthy subjects, and whether the impairment could be dependent on the duration and/or the severity of the previous attack. Patients suffering from migraine with aura showed cognitive impairment while in case of migraine without aura impairments were not detected; duration of attack was equal in both groups. During interictal period there was no impairment in cognitive functions detected in both groups of migraine patients (19).

In 1999 Gelman et al opened a new aspect in neuroimaging, demonstrating results of MR imaging of brain and describing iron concentration in brain of healthy volunteers. Authors suggested that increased level of iron in the brain is closely related to age and could be involved in the pathophysiology of several neurodegenerative disorders (8). Two years later Welch et al suggested that iron homeostasis in the brain of migraine sufferers is impaired and lesion volume could be related to frequency of migraine attacks (32). In subsequent investigations increase in iron level in the gray matter of aging brain was revealed. It was suggested that increased iron level is detected also in a range of chronic neurological disorders. From this perspective, it is important to understand firstly the normal cellular

and anatomic age-dependant distribution of iron in the brain, followed by evaluation of abnormal iron deposition later (29). Unfortunately, it is still difficult to differentiate normal and abnormal iron level in a certain patient. Susceptibility–weighted MRI is applying to be very promising investigation method differentiating calcium from iron and hemorrhagic products in the aging brain, where deposition of calcium and iron is observed normally (30, 31).

May A (2006) using the voxel-based morphometric analysis of the structural MRI scans did not detect any structural changes in brain of migraine patients as it was suggested before, although changes were detected in tension type headache sufferers. Using single photon emission computed tomography (SPECT) no blood flow changes were discovered in migraine without aura during attack in contrary to migraine with aura (17). Later, the majority of trials performed did not support the findings on structural integrity of the brain in case of severe migraine. Lipton at al proposed to conceptualize migraine as a chronic episodic disease (or chronic progressive disease in some patients) due to detectable damage in the brain- subclinical posterior circulation stroke and diffuse white matter lesion, with lesion volume increasing with the frequency of migraine attacks (15).

Schoonman et al in 2008 using 3- Tesla magnetic resonance angiography (MRA) investigated diameter and blood flow in the basilar and internal carotid arteries during migraine attack and made a revolutionary statement- migraine headache is not associated with cerebral and/or meningeal blood vessel vasodilatation as it was previously widely believed (27).

Detectable brain lesions in migraine

Kruit et al in review in 2010 have clearly stated that migraine is not only a risk factor for brain lesions, but it is a direct cause of brain lesions. In magnetic resonance imaging study CAMARA (Cerebral Abnormalities in Migraine, an Epidemiological Risk Analysis) authors discovered that in patients with migraine (especially, migraine with aura) had significantly higher incidence of subclinical brain damage. Female gender was recognized as a specific risk factor of white matter lesion. Migraine patients also had higher incidence of brainstem hyper-intense lesions in comparison to healthy controls. In comparison with matched by age and gender controls , migraine patients over 50 years were found an increased iron level in areas that are believed to be involved in central pain processing- putamen, globus pallidus and red nucleus. A causal relationship between migraine and brain lesions was identified in patients with frequent attacks (one or more attacks per month) and/or long lasting migraine duration. Authors also concluded that impairment of cortical brain function is detectable in migraine sufferers that can lead to cognitive changes not only during attack, but also interictally. In the light of these findings, importance of migraine prevention proves to be much higher than considered before (12).

CONCLUSIONS

Migraine is very common paroxysmal neurological disorder for long time being perceived as severe headache with no brain lesions. Recent achievements in neuroimaging have revealed migraine as disabling disease with increased risk of deep white matter lesions, subclinical posterior circulation infarcts and increased iron accumulation in brain. The yield of neuroimaging in diagnosis of migraine is little when to exploit it only for exclusion of secondary headache or with the aim of reassurance of patients. The role of invasive methods, such as lumbar puncture and angiography with contrast, remains to be consistently high in diagnosis of coexisting illnesses, such as subarachnoid haemorrhage and pulmonary and cardiac right-to-left shunts. In the future we can expect neuroimaging procedures being able not only to exclude secondary headache, but also giving decent affirmation of migraine, characteristic changes in brain in cellular and even subcellular level, thereby saving common health expenditures and time of every involved participant, either patient or medical personnel. When assessing migraine as a kind of neurodegenerative disorder that can lead to brain damage, it is extremely important to diagnose it early and prevent proactively.

Conflict of interest: None

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PROBLEM-SOLVING ARTICLE

Evaluation of Oral Therapeuthical and Surgical Treatment Needs among Retirement Age Population in Different Countries

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Summary
Oral health in connection with quality of life is affected by such functional factors as dental decay and its complications, untreated tooth roots, oral mucosal diseases and inflammations, precancerous diseases, cancers, pain in temporomandibular joints, xerostomia and partially or fully edentulous jaws. It has been noted in literature that among retirement age population the number of remaining teeth has increased and the number of untreated decayed teeth in developed countries for the last 20 years has decreased. Despite this fact the need to improve measures of oral health remains actual in this age group due to increasing prevalence of diagnosed oral diseases and number of extracted teeth and roots. Oral health indicators among retirement age population living in nursing homes in such countries as Canada, USA, UK, Finland, Denmark, Germany, Turkey, Brazil, Australia and Lithuania differ from the same age group indicators among self-dependent old people able to take care of themself. Oral health indicators of nursing homes residents in many countries are significantly worse than oral health indicators of the corresponding age group population. The proposed evaluation data of oral hygiene, periodontal status, DMF-T index, quality of existing and needs of new prosthodontics as well as oral mucosal disorders among retirement age population provides an important insight into therapeutic and surgical treatment provision in different countries.
Key words: oral care of old people, oral surgery, dentistry, prosthodontics

INTRODUCTION

Age is inescapable biological condition at which the human body and dental status is undergoing significant changes, however, environment in which it occurs influences the human health in its own way. Each country develops its own experience how to efficiently attract finances to the health care system, preserve and promote the health of the population. Health care model in different countries depends on regional traditions, national historical development and state role in social care regulations (18,20,22). Indicators of oral health among retirement age population living in nursing homes differ from the same age group indicators among self-dependent old people able to take care of themself (24,26,36). Oral health in connection with quality of life is affected by such functional factors as dental decay and its complications, untreated tooth roots, oral mucosal diseases and inflamations, precancerous diseases, cancers, pain in temporomandibular joints, xerostomia and partially or fully edentulous jaws. Physiological and social aspects play significant role. The typical feature of the elderly is contradiction between the subjective treatment requirements and the objective treatment needs (7). Frequently the reason of poor oral health condition among old people is chronical diseases and seriously exhausted nature which influences overall quality of life (22,24). To assess and compare oral health condition of retirement age population in the world and in Latvia generally accepted oral health characteristics are used: oral hygien**e**, DMF-T index (D-decayed, M-missing, F-filling number of teeth), health status and visible changesoral mucosa and periodont, quantity and quality of prosthodontics.

Evaluation of oral hygiene and periodontal status of retirement age population

The most popular oral hygiene method in many countries is teeth cleaning using only a toothbrush and toothpaste. Regular teeth cleaning among old people is less frequent than in general population (20,26). Although oral health care is available for all age people in developed countries, older people have less possibilities to use it (22,26,35). In the absence of state financial support and co-payment system for many old people the oral care is not available (23). Due to the physiological aging process old people have serious problems to maintain good oral hygiene (2,8,9), particulary among people with hand movement disorders, joint pain and stiffness, poor vision, cerebrovascular and mental disorders, resulting in poor oral hygiene and increased plaque amount on teeth and dentures surfaces (13,24). Unsatisfactory oral hygiene causes periodontal problems leading to progressive bone loss, exposed root surfaces, teeth movement, root decay and with time these teeth will be lost (19,23). Oral health studies have revealed a high parameters of teeth plaque and gingival index in complex with high

caries and root caries incidence (2,8,20,26). Microscopic analysis of mouth swab scrapings in many nursing homes residents revealed fungal, staphylococcal and colibacterial infections (10,41). The main predisposing factors of oral candidiasis are ill-fitting dentures and poor oral hygiene (13,27,35).

In such economically and socially developed country as Canada 90% of nursing homes residents take care of teeth regulary and 68.5% do it by themselves. However, almost all with few exceptions had dental plaque and food remains on teeth, 48.7% of them had periodontal diseases (gingivitis, calculus, periodontal pockets), so more than 90% of old people need hygiene instruction and professional teeth cleaning and calculus removal (1,9,22). In Finland oral hygiene of old people continuously living in nursing homes was assessed as poor, only in 19% of cases hygiene of removable dentures was assessed as good (21). In Turkey old people from nursing homes mainly need regular oral hygiene and nursing home's staff needs special education in maintenance of oral health (20). In Germany (27) oral health and oral hygiene of old people living in nursing homes was unsatisfactory, it was affected by social and economical factors. Among people with poor oral and denture hygiene frequently is observed denture stomatitis (10,13,16). In many cases instruction of denture hygiene does not cover routine care responsibilities of nursing home's staff (9,13,24). Patients and nursing home staff was not properly educated in oral hygiene measures (13,24,28,35).

Studies performed in Latvia (3,5,17,39) revealed that oral health condition of retirement age population is not significantly different from that mentioned in literature (5,17,40). In 2005 study (6,9) in Latvia in 87% of retirement age population was observed soft dental plaque which is the main cause of dental and periodontal diseases (3), but in 1993 study in 65 – 74 years age group 100% of population were observed periodontal problems (37). In 2005 study (5,19) investigating periodontal status of old people in comparison with 1993 study was found out that periodontal health in general did not improve and in 2005 healthy periodontal tissues was observed only in 4.1% of examined 64 – 74 years age people (5,19).

Evaluation of DMF-T index and its parameters among retirement age population

In different countries oral health status among retirement age population is different, it is most closely related to the national socio-economical situation (9,22). WHO (World Health Organization) studies (23) indicate that in developed countries DMF-T index of old people is 22 and more (23). DMF-T indices in: United States – 21.9 (38), Canada – 24.86 (1), Australia– 24.7 (33), Turkey – 29.3 (36), Brazil – 30.2 (25), Fiji islands – 23 (8). In European countries this index is similar: Germany – 22.0 (27), Denmark – 16.7 (18), Croatia – 27 (28), Lithuania – 25.63 (42), Italy – 19.82 (2).

In Latvian residents over 60 years of age the mean DMF-T index in 1993 was 24.92 (6,37), but in 2005 study it was 24.84 (5). In 2010 this index was worse (17). DMF-T index among retirement age population which regulary visit dentist was 25.0 for men and 25.9 for women, but in Latvia Zemgale region nursing homes DMF-T index was 25.5 for men and 27.5 for women, in Latvia Kurzeme region nursing homes it was 28.3 for men and 29.2 for women (17,39,40). The main DMF-T indices components were the number of lost and decayed teeth, from which the main part was root decays and roots which must be extracted. In Latvia oral health parameters among retirement age population are worse than in the most European countries (17,39,40). In developed countries during last 20 years the number of remaining teeth among retirement age population is increased but the number of untreated decayed teeth is decreased (1,18,22,26). Nevertheless the need to improve measures of oral health remains actual in this age group (1,9,23) because of increasing prevalence of diagnosed oral diseases (1,13,42). There is observed high prevalence of dental and root caries among old people in such countries as Brazil, Turkey, United Kingdom, Poland, Lithuania (12,20,23,24,25,42). The data about root caries revealed that exposed root surfaces in combination with poor general health and drugs use could significantly increase risk of root caries development in old people population (8,22,28,42). The main component of DMF-T index in old people is the number of lost teeth the reasons for this are widespread dental and root decay and periodontal diseases (12,19,20,26).

Evaluation of quality of existing prosthodontics and needs of new prosthodontics among retirement age population

In many countries among older population is observed complete teeth lost (21,22,42). The epidemiological studies (20,21,32) revealed also that people with low income, low social status and low education more often is observed complete teeth lost in comparison with people with higher income, social status and education (12,24,36). In industrial countries many retirement age people have removable dentures, epidemiological data in many countries (14,22,35) revealed that one third to half part of old people use complete removable dentures and untill three quarters of old people use complete and/or partial removable dentures (14,20,24,34,35). The number of retirement age fully edentulous patients in literature is pointed withlarge degree of difference: in France – 26.9% (34), in USA – 43.1% (26), in Scotland – 51.7% (32), in Brasil – 74.9% (12). In literature amount of denture's users is not devided by gender but there is specified that the number of complete denture users increases with age: in Belgium – 47.0% (9), in Poland – 25.0% (23), in Lithuania – 14.0% (42), in Turkey – 11.9% (36). Among Latvian residents the need of new dentureswas 60.4% (30). The need of new dentures among Zemgale region nursing home residents was found in 98.4% of cases and in Kurzeme region nursing homes residents – 97.6% (17,40). Among retirement age population who regulary visit dentist 60.9% needed new dentures (17,29,30,39). It is often claimed that aesthetic characteristics of dentures is not

so important for old people and that functionality of dentures is much more important than psychological and social aspects (7,9,34). Depression and dementia is common among older generation (9,26), so oral health often is neglected. Literature indicates non-compliance of dentures to its functional quality and wherewith in many cases patients dont use their removable dentures: in USA – 5.2% (26), in France – 12.6% (34), in Brasil – 42.6% (12). Despite the fact that part of unsuitable denture users assess their dentures as good (29), often they have denture caused oral health problems. This can be explained by the fact that patients are modest in their demands (37) and often because of financial reasons they can not afford to make a new dentures (7,14,20,38). The status of general health among old people, manual dexterity, problems of self care contribute to poor oral hygiene indices (26,35,40). The typical feature of the elderly is contradiction between the subjective treatment requirements and the objective treatment needs(7,22,38). Oral and dental health of old people living in nursing homes significantly differs from the same age group self-dependent population in Latvia (17,39,40), similar differences is observed in such countries as Norway (14), Sweden (38), Canada (1), Turkey (20). Oral health of old people living in nursing homes is different in many countries, it depends on national socio-economical situation (36). The difference of oral health indicators among Latvian residents over 60 years of age is associated with elimination of dental care in nursing homes in Latvia (39,40).

Oral mucosal changes and diseases among retirement age population

Oral mucosal changes and diseases related to human aging frequently are complex and may adversely affect the quality of life (11,13,31). In literature, as main health problems are noted oncological and precancerous diseases, inflamatory vesiculo-erosive diseases and candidiasis (10,11,15,31). WHO data (23) and literature data revealed that in approximately one half of humans over 65 years of age is found at least one oral mucosal lesion (10,11,35). Oral mucosal diseases in the world population have a high degree of diference: in Germany – 19.55 % (31), in Hungary – 10.14 % (16), in China – 12 – 26 % (23), in Greece - 47 % (35), in Turkey – 40.7 % (10), in Chile – 53 % (11), in Thailand – 83.6 % (15). In 2010 study performed in Latvia oral mucosal changes and diseases among retirement age population was found in 41.9 – 90.1%, worse oral health indicators were observed in residents of nursing homes (17). The most frequent oral mucosal lesions among retirement age population is denture stomatitis, xerostomia, traumatic ulcers and varices (11,15,35), among precancerous lesions most frequent is leukoplakia and oral lichen planus (11, 15, 31). Another more frequently in literature cited oral mucosal lesions are hemangiomas, hyperplasias, cheilitis angularis, atrophy of mucosa, fissured tonque, smoking melanosis and stomatitis, aphtous stomatitis, median rhomboid glositis, cheilitis actinica (11,15,35). In study performed in Latvia most frequently observed oral mucosal lesions were coated tonque, atrophy of oral and tonque mucosa, varices and cheilitis angularis (17,39). Approximatelly one third part of retirement age population has denture stomatitis arised from poor denture hygiene and usage of these removable dentures around the clock (10,12,15,35). In Finland study (21) 25% of old people with dentures had denture stomatitis and 28% - cheilitis angularis. In Turkey denture related lesions were found in 36.4% of retirement age people with old dentures and among patients with diabetes and increased risk of development of dentue stomatitis and denture hyperplasias (10). In Thailand study (15) in age group over 60 years with removable dentures significantly higher was observed oral mucosal lesions (62.7%) than among old people without removable dentures (28.5%) and people with fixed crowns or bridges (8.8%). In Greece study denture related stomatitis was observed in 17.2% of old people (35), and in Chile – 22.3% (11). Three most common denture related oral mucosal lesions are traumatic ulcers (22.6%), denture stomatitis (14.3%) and cheilitis angularis (4.8%). Denture related oral mucosal lesions more frequently was observed in complete removable dentures users (46.3%) than among partial dentures users (40.8%) (11). Oral candidiasis commonly affect immunosuppressed individuals in all ages but among old people living in nursing homes fungal infections are especially prevalent (10,23,41). Prevalence of oral fungal infection among retirement age people living in nursing homes is associated with poor oral hygiene and negligent care of dentures (4). In Poland study the highest prevalence of Candida species in oral mucosa was found among 56 – 70 years old people (35%) and among 71 – 92 years old group (74%) with or without removable dentures (41). Oral and dental health among old people living in nursing homes significantly differ from the same age people oral health in Latvia. The difference of oral health indicators among Latvian residents over 60 years of age is associated with elimination of dental care in nursing homes in Latvia (40).

CONCLUSIONS

1. World Health Organization (WHO) studies indicate that in developed countries DMF-T index of old people is 22 and more. The mean DMF-T index among retirement age population in Latvia is 24.84 (2005 study).
2. In many countries similar to Latvia the main component of DMF-T index among retirement age population is the number of lost teeth.
3. In Latvian retirement-age population which regulary visit the dentist quantity indicators of prosthodontics with partial dentures do not significantly differ from Europien retirement-age population's dental prosthodontics indicators.
4. Literature indicates non-compliance of dentures to its functional quality and wherewith in many cases patients dont use their removable dentures (difference from 5.2% to 42.6%).
5. Among retirement age population is observed one or more oral mucosal lesions or diseases (data variation from 10.14% to 47%).

Conflict of interest: None

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CASE REPORT

Primary Bronchus-Associated Lymphoid Tissue (BALT) Lymphoma

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Summary
Bronchus-associated lymphoid tissue (BALT) lymphoma is a rare extra-nodal marginal zone lymphoma representing 0.5% of lung tumours. In order to share experience in the management of BALT lymphoma, we report a well-investigated case with unusual features as complex familial oncologic history and synchronous benign thyroid disease. The patient was a 44-year-old woman with prolonged unspecific respiratory complaints. By computed tomography (CT), bilateral atelectasis-like pulmonary lesions were found in the 3rd, 5th, 6th and 8th segments of the right lung and in the 3rd segment of the left lung. We performed diagnostic video-assisted wedge resection of the 5th segment of right lung. Pathologic and immunohistochemical examination yielded the diagnosis of BALT lymphoma. After staging by bone marrow biopsy and abdominal CT, chemotherapy was recommended by the oncologic council.
Key words: bronchus-associated lymphoid tissue, BALT lymphoma, lung tumour.

AIM OF THE DEMONSTRATION

We report a well-documented rare tumour with a complex familial oncologic background in order to increase awareness about BALT lymphoma and the related role of thoracic surgery.

CASE REPORT

A 44-year-old woman who was a milkmaid in Latvia consulted doctor complaining about dry cough and sweating in stress situations. The symptoms lasted for 14 months. A year ago, patient underwent right hemithyroidectomy for solid "cold" nodule, proved by ultrasound examination and scintigraphy. At that time, the patient recalled pneumonia-like illness few weeks before the surgeon consultation and chest X-ray scan showed atelectasis of the 6th segment of right lung. In the removed thyroid tissue, nodular colloid goiter was found by pathology (Fig.1). Postoperative levels of TSH, FT4, FT3 and calcium were within normal limits. However, chest CT showed persistent lesion.

The patient had smoked for 20 years. There was no history of allergy, autoimmune disease, other lung disease or serious infections. The family oncologic history was remarkable for several cases of lung cancer, haematologic neoplasms and extra-pulmonary solid tumours.

At present, physical examination was unremarkable. Laboratory data including blood tests, renal and liver function tests were within normal limits. Chest CT (Fig.2) revealed multiple bilateral pathological foci in lung parenchyma. The 3rd, 5th, 6th and 8th right lung segments and the 3rd left segment were affected. Fibre-optic bronchoscopy was performed as well. Diagnostic video-assisted wedge resection of the 5th segment of the right lung yielded sufficient amount of tissue for diagnostic investigation. Pathologic and immunohistochemical examination demonstrated low-grade B-cell BALT lymphoma (Fig.3). In order to estimate the extent of tumour spread, the patient underwent abdominal CT and a bone marrow biopsy. Chemotherapy was recommended by oncologic council.

DISCUSSION

BALT lymphoma is a rare low-grade lymphoma, mostly of B-cell origin as in the presented case. It represents 1% of lymphomas (2) and 10% of MALT lymphomas (7). The published data mostly are limited to case reports or small groups comprising less than 22 patients (1). The development of BALT lymphoma can be triggered by chronic antigen stimulation (6) including Sjogren's syndrome (6,7), tuberculosis (7), bronchectasis, *Mycobacterium avium complex* infection (5), rheumatoid arthritis (7), thyroid cancer and previous pneumonia (9). Atopic dermatitis has been described as the only source of antigen stimulation in a young man (8). In our patient, several causal relationships are possible including occupational factors and exacerbation of smoker's bronchitis. The thyroid disease seems pathogenetically unrelated as it lacked inflammatory component.

The disease can be clinically silent or cause unspecific general (3,11) and respiratory symptoms (6,7) including productive cough and dyspnea (4). The patients' age ranges 22-80 years (6,8). The documented clinical course can be as long as 7 years (11). Thus, the age characteristics and clinical course of the disease in our case are typical. Although the delay in diagnosis was not desirable, it might be innocuous in case of BALT (10,11). Radiologically, BALT lymphoma can represent single mass, occasionally large or associated with pleural effusion (4). Bilateral disease, nodules and air space consolidation with or without air bronchogram are frequent findings (3,6,8). Positron emission tomography can reveal additional foci (4). The diagnosis in published

cases has been reached by examination of tissue obtained by bronchoscopic biopsy (4), transbronchial lung biopsy (3,7) and open or thoracoscopic lung biopsy / wedge resection (3,5,7).

Variety of treatment options has been described, including surgery (10), chemotherapy either as monotherapy or by combined regimen (7), radiotherapy (6), regular (10) or extended (4) rituximab treatment and even observation (10). Only few patients have experienced disease progression (10). The infrequent T-cell BALT lymphoma has shown unfavourable course (3). In conclusion, BALT lymphoma is potentially curable lung tumour entering the differential diagnosis of single, multiple and bilateral lung lesions. Thoracic surgery can be recommended both for the diagnostic and curative value.

Conflict of interest: None

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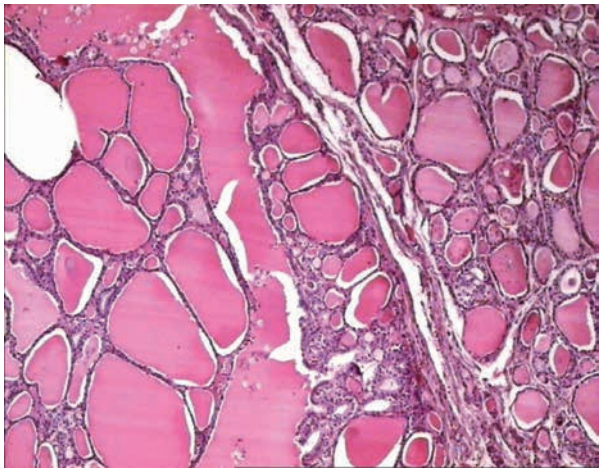

Fig. 1. Nodular colloid goiter lacking inflammatory infiltrate. Haematoxylin – eosin, original magnification 50x.

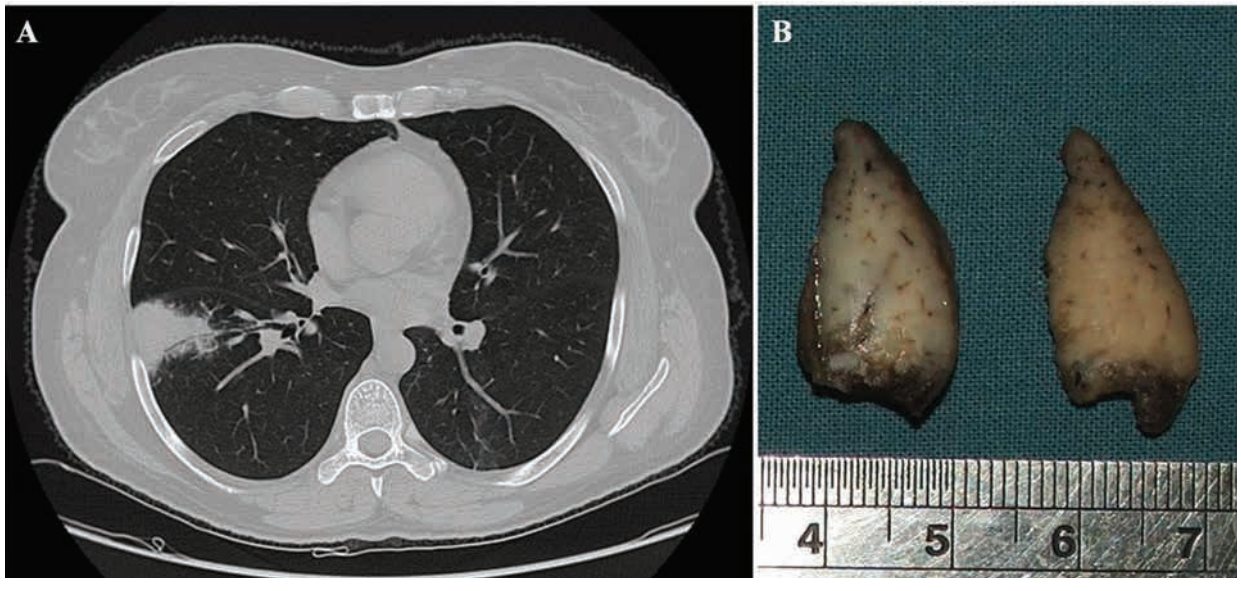

Fig. 2. Gross changes in the lungs. A, Computed tomography scan of the lungs. Note the peripheral pathological focus. B, Gross view of the resected tissue showing consolidation.

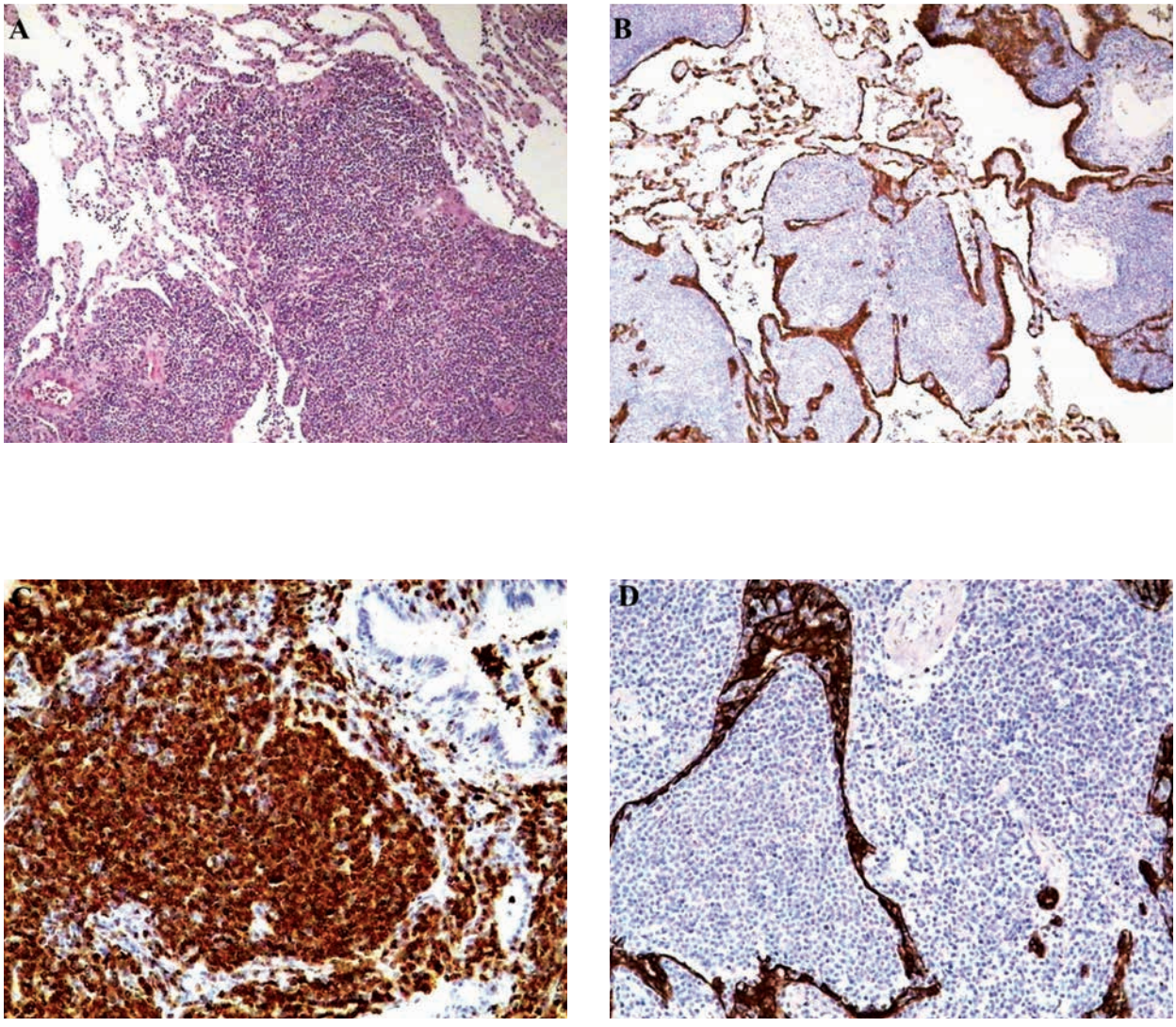

Fig. 3. Bronchus-associated lymphoid tissue lymphoma. A, The tissue structure, characterised by dense monomorphic infiltrate of lymphoid cells. Haematoxylin-eosin, original magnification 50x. B, The tumour spread along lymphovascular bundles. Immunoperoxidase, anti-cytokeratin AE1/AE3, original magnification 50x. C, The monoclonal cellular composition of B-cell origin. Immunoperoxidase, anti-CD20, original magnification 100x. D, Lymphoepithelial lesion. Immunoperoxidase, anti-cytokeratin AE1/AE3, original magnification 100x.

CASE REPORT

Management of *BRCA1* Mutation Carrier with Breast Cancer

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Summary
BRCA1 gene mutation carriers face high lifetime risk of breast cancer therefore specific diagnostic features should be considered and individualized treatment strategies should be applied. For *BRCA1* mutation carriers with already developed breast cancer, extensive surgery – bilateral mastectomy – could be indicated due to the cancer risk in the contralateral breast.
Key words: breast cancer, *BRCA1* mutation, prophylactic mastectomy.

AIM OF THE DEMONSTRATION

In order to increase awareness about the problems and solutions treating *BRCA* mutation carriers, we present *BRCA1* positive breast cancer case with characteristic management challenges related to the young age of patient and biological properties of *BRCA1*-related tumour. The individualized surgical and chemotherapy approach is shown as well.

CASE REPORT

A 27-year-old female complained about palpable lump in her right breast during lactation period. Ultrasound (US) imaging showed benign-appearing mass, 1.0x0.6 cm (Figure 1). Fine needle aspiration cytology (FNA) of the breast lump revealed chronic inflammation only. The patient was consulted again after 7 months. The lump had increased slightly, reaching size of 1.2x1.3 cm. Core biopsy by 14-gauge needle revealed triple-negative (i.e. negative for estrogen and progesterone receptors, and HER2 protein expression) medullary breast cancer with high proliferative activity. Unfortunately, the patient was in the first trimester of her 3rd pregnancy at that time. The family history disclosed that her mother had had breast cancer at age 41. By genetic testing, pathogenic *BRCA1* mutation 5328insC was identified in the blood.

Medical termination of pregnancy was recommended by multidisciplinary medical council because of necessity of neoadjuvant chemotherapy. It was performed shortly before cancer surgery. Patient underwent sentinel lymph node biopsy and right mastectomy. The final pathology revealed medullary cancer (Figure 2) with high grade nuclear morphology, associated with ductal carcinoma in situ. The tumour measured 1.5x1.0x1.0 cm. Dense infiltration of lymphocytes and plasmocytes was observed in cancer stroma. By immunohistochemistry intensive membranous expression of E-cadherin, intense nuclear expression of p53 protein (Figure 3), high proliferation fraction (Figure 4) by Ki-67 (58%) and intense pan-cytokeratin AE1/AE3 expression were found. The molecular subtype was triple-negative. Resection margins were free from cancer. Harvested eight axillary lymph nodes were negative for malignancy. The diagnosis of medullary breast cancer, pT1N0M0R0, stage I, was made. Adjuvant chemotherapy after surgery was advised by multidisciplinary council and four courses of Cisplatin and Doxorubicin were administered.

One year after cancer treatment contralateral risk-reducing mastectomy with bilateral immediate expander/ implant reconstruction was performed (Figure 5). No cancer was detected in the final pathology of her left breast. During 30 month follow-up, she is disease-free and fairly satisfied with the cosmetic outcome of reconstructive surgery.

DISCUSSION

Accurate diagnosis of breast lesions is crucial for appropriate treatment planning and patient consulting. FNA is useful for initial evaluation of palpable breast masses as it is cheap and fast. However, findings from cellular samples are limited and the rate of false-negative FNA diagnoses is high. Because of these limitations with FNA, core needle biopsy was developed. The main advantage of core biopsy is enabling of histological diagnosis, which is vital to the planning of subsequent surgery and treatment. Stereotactic core biopsy using a 14-gauge needle is widely accepted to be sensitive (90.5%) and specific (98.3%) in diagnosing breast masses, compared with 62.4% and 86.9%, respectively, for FNA (5). Therefore, core biopsy must be the first choice for early establishing of diagnosis and adequate management of *BRCA1/BRCA2* mutation carriers with breast lumps. The importance of this recommendation is further emphasized by significantly more frequent occurrence of medullary cancer in *BRCA1* mutation carriers (7) as this type of breast cancer is defined by pushing border and intense reactive infiltration (7) posing difficulties in US and FNA evaluation.

Current treatment guidelines for early-stage breast cancer recommend local therapy, i.e. surgery with or

without radiotherapy, and adjuvant systemic treatment (1). *BRCA1/BRCA2* mutation carriers are at high risk of contralateral breast cancer (CBC). According to Pierce *et al.* (3) the rate of CBC at 15 years was 39% in *BRCA1/BRCA2* carriers compared with 7% in sporadic breast cancer. The 10-year CBC risk (2) was also higher for women younger than 50 years at first breast cancer diagnosis (31%) compared with those diagnosed after age 50 years (23.5%). These data suggest that a more extensive surgical procedure as bilateral mastectomy could be considered for mutation carriers with a diagnosis of breast cancer in order to reduce CBC risk. Prophylactic surgery remains the most effective modality for reducing cancer risk and mortality. Prophylactic bilateral mastectomy reduces the breast cancer risk in *BRCA1/BRCA2* mutation carriers by 90% (4). Compared with patients who had breast conservation or unilateral mastectomy, those who chose mastectomy of the affected breast and contralateral prophylactic mastectomy of the unaffected breast did not report diminished quality of life or elevated distress (6).

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Conflict of interest: None

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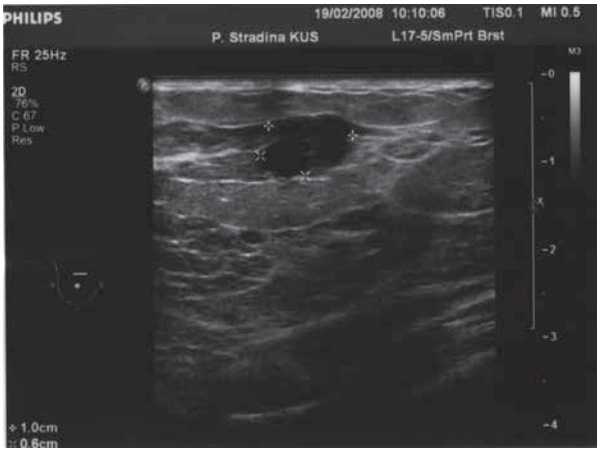

Fig. 1. Ultrasound imaging of the right breast revealing benign-appearing mass, 1.0x0.6 cm.

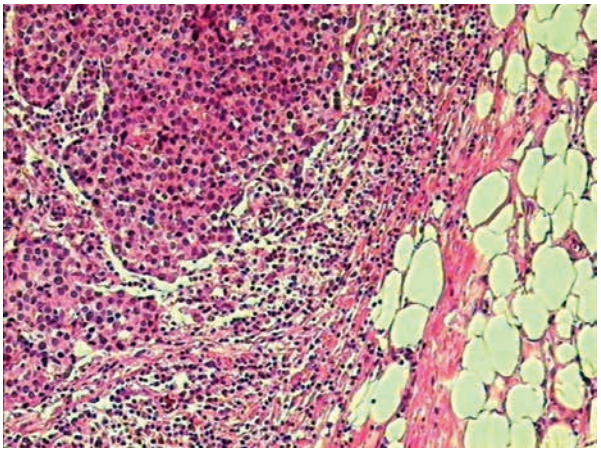

Fig. 2. Medullary breast cancer showing syncytial growth and pushing border. Haematoxylin-eosin, original magnification 100x.

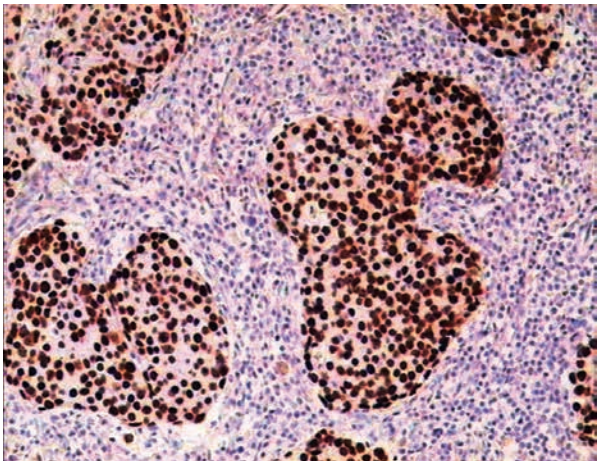

Fig. 3. Intense nuclear expression of p53 protein in cancer cells. Immunoperoxidase, original magnification 100x.

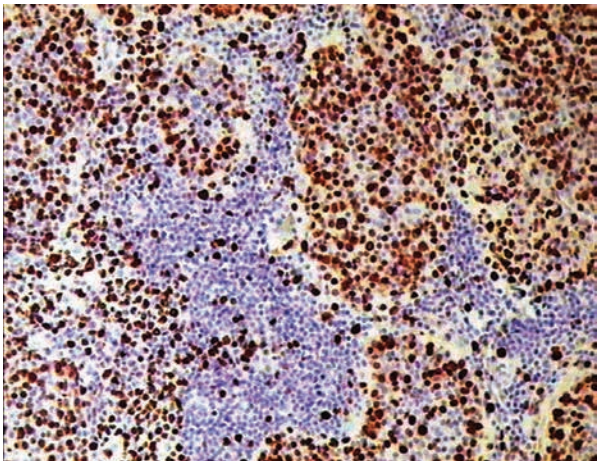

Fig. 4. High proliferative activity in cancer cells by immunohistochemical visualisation of Ki-67 protein (clone MIB-1). Immunoperoxidase, original magnification 100x.

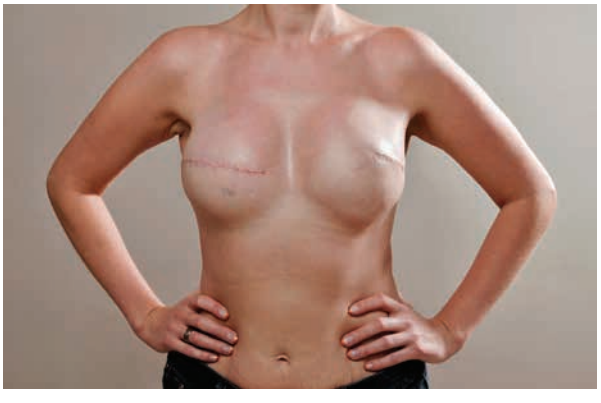

Fig. 5. The outcome after curative mastectomy and contralateral risk-reducing mastectomy with bilateral expander/ implant reconstruction.

CASE REPORT

Primary Adenocarcinoma of the Appendix

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Summary
Primary adenocarcinoma of the appendix is exceedingly rare type of malignancy with the incidence of 0.4 cases per 100,000. It composes less than 0.5% of gastrointestinal malignant neoplasms. Frequently, the diagnosis is reached only after histological examination of surgically excised appendix due to suspected inflammation. Despite the low survival rate in case of primary appendix tumours aggressive therapy is necessary to obtain long-term survival. We present a well-documented case of instant neoplasm evaluated at our institution. To the best of our knowledge, no such detailed studies have been carried out before in Latvia.
Key words: adenocarcinoma, vermiform appendix, mucinous neoplasm.

AIM OF THE DEMONSTRATION

We present thoroughly documented case of primary adenocarcinoma of the appendix along with imaging studies in order to demonstrate an exceedingly rare case of malignant tumour.

CASE REPORT

Seventy nine-years-old female was admitted to the hospital due to dull pain in the right flank and febrile temperature. During her disease history, this was the third hospitalization within a year. Six month ago she was admitted to urology department with the same clinical symptoms and signs including blunt pain in the right side and fever. Right side lumbotomy with drainage was performed then due to retroperitoneal abscess. Two weeks later, re-operation was needed along with drainage.

During the third hospitalization the computed tomography (CT) scan revealed 9.5x3.0x3.0 cm large abscess cavity in the retroperitoneal space along with periappendicular infiltration. The laboratory investigations demonstrated haemoglobin level as low as 86 g/L and markedly increased C-reactive protein (CRP) level up to 269.9 mg/L. No other abnormalities were found by the laboratory tests. At first, relumbotomy was done and abscess cavity was opened. Bacteriological examination disclosed *Enterococcus spp.* sensitive to Ampicillin, Vancomycin and Linezolid. The relumbotomy wound healed secondarily. A week later appendix was surgically removed through lower median laparotomy access. During the operation it was observed that the appendix was placed close to retroperitoneal space, it had unusual high density on palpation and lacked gross signs of acute inflammation. Fistula tunnel was found at the end of the apex. The postoperative recovery was slack. The CT of the abdominal cavity and retroperitoneal space was repeated on the 7th postoperative day and showed no fluid collection. The laboratory tests demonstrated only slightly elevated CRP level up to 50.6 mg/L. Histological examination of the appendix revealed invasive high-grade mucinous adenocarcinoma (Figure 1). The tumour invaded through the serosa at the apex. The final estimate of the cancer spread was pT4G3R0. The appendicular mucosa was completely replaced by villous adenoma extending to the resection line and showing high-grade dysplasia. The presence of precursor lesion confirmed the true cancer origin in the appendix. Oncologic council concluded any chemotherapy or extended surgery is not necessary. The dynamic surveillance was required instead. No metastases were observed during the hospitalization and surveillance of 1 year. The colonoscopy and CT scan were performed year later and did not reveal any malignant tumour.

DISCUSSION

Primary appendicular neoplasm was the first described by Berger in 1882 (5). The primary carcinoma of the appendix is a rare malignancy accounting for 0.4-1.0% of all gastrointestinal tumours, while primary adenocarcinoma of the colon is widespread (13). The rarity of the instant neoplasm has also embarrassed the scientific studies (1,2). To meet the requirements of evidence-based research, in all cases the diagnosis of appendicular adenocarcinoma has been verified by the pathologists performing formal histology after the appendectomy (14). Mucinous adenocarcinoma is one of the three histological subtypes of the primary appendicular adenocarcinoma including also colonic type and signet ring cell cancers (13). This tumour arises in pre-existing adenomas as in our case (3). In contrast, the carcinoid (called also gastrointestinal neuroendocrine tumour) is the most common neoplasm of the appendix constituting 90% of all appendicular primary tumours.

Patients with appendicular malignancies usually present with the symptoms of acute appendicitis (9). There are no specific symptoms of the appendicular tumour (4). In accordance with the published studies, the appendicular neoplasm was established preoperatively in none of

cases (6,12). The sex distribution is usually equal (7).
Appendectomy is appropriate for the small tumours (pT1), usually found incidentally, measuring less than 2 cm and lacking mesoappendiceal involvement (4). On the other hand, several clinical studies have demonstrated better survival rate after the right hemicolectomy in comparison with appendectomy (9). For primary appendicular tumours greater than 2 cm, involving the mesoappendix or base of the appendix, right hemicolectomy should be considered (5,11). Overall 5-year survival is 44% for appendicular mucinous tumours, 52% and 20% for the colonic subtype and for the signet ring cell subtype, respectively (10). The general prognosis is comparable to the colonic cancer (8).
In conclusion, primary adenocarcinoma of the appendix has diverse clinical manifestations. Surgeons should keep in mind the possibility of primary appendicular malignancy when managing patients with suspected acute appendicitis. To improve the outcome, right hemicolectomy should be recommended.

Conflict of interest: None

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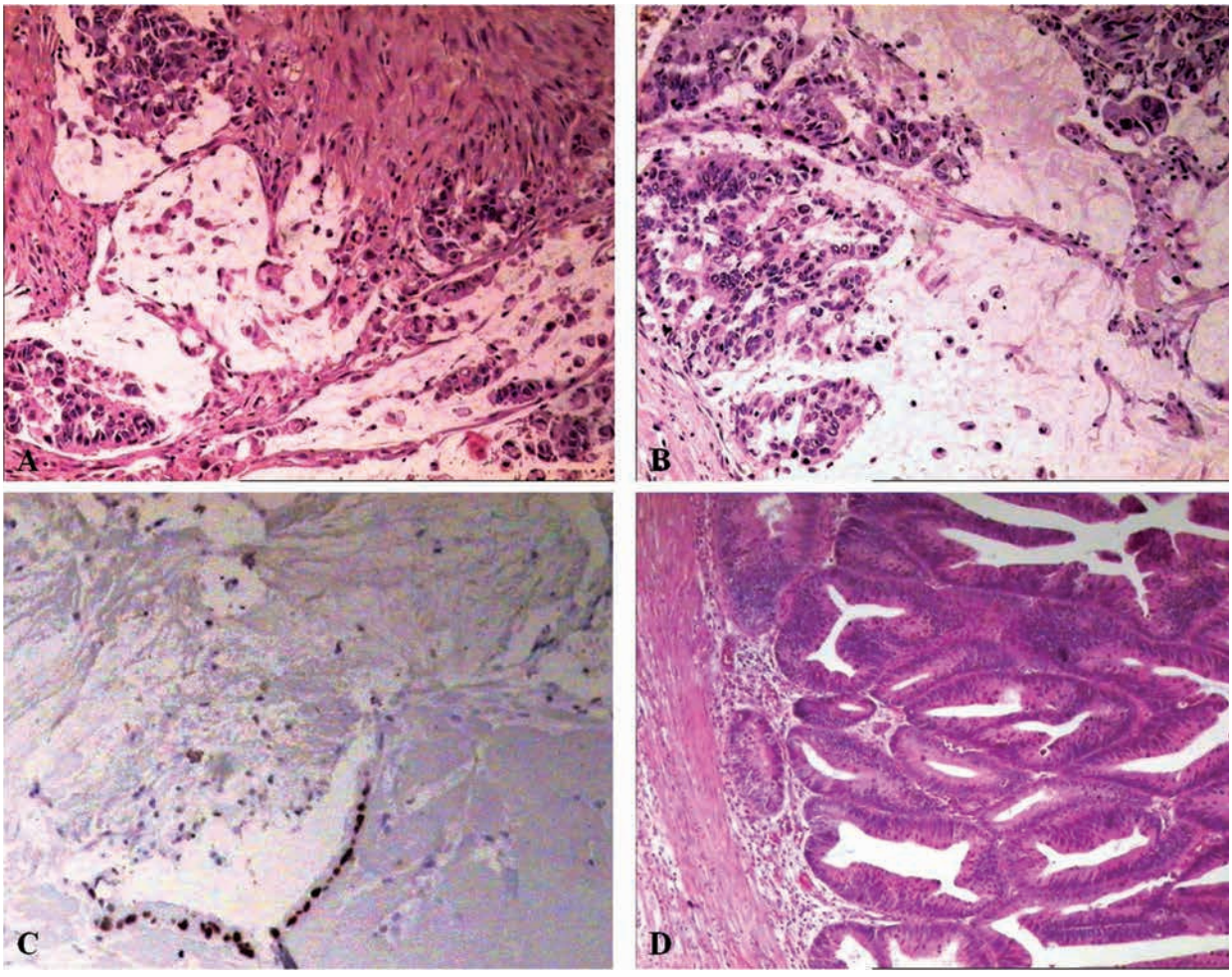

Fig. 1. The histologic structure of the appendicular adenocarcinoma. A, Invasive growth. Haematoxylin-eosin (HE), original magnification (OM) 100x. B, Marked nuclear atypia in the tumour cells. HE, OM 100x. C, Nuclear expression of CDX2 in the neoplastic cells. Immunoperoxidase, anti-CDX2, OM 100x. D, Villous adenoma replacing the appendicular mucosa. HE, OM 100x.

CASE REPORT

Incidental Primary Non-Hodgkin's Lymphoma of the Prostate in a Patient with Prostate Cancer: A Case Report

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Summary
Primary lymphoma of the prostate is uncommon; it is extremely rare combination of adenocarcinoma and primary Non-Hodgkin's lymphoma of the prostate. We report a case of 69-years-old man who was treated with a radical surgery.
Key words: prostate Cancer, Primary Non-Hodgkin's Lymphoma.

AIM OF THE DEMONSTRATION

Patients with prostate cancer are everyday practice among urologists. However incidentally secondary malignancies of the prostate are detected. Primary Non-Hodgkin's lymphoma of the prostate is very rare with frequency less than 0.09% of all prostate neoplasias [2]. But the combination of non-Hodgkin's lymphoma with adenocarcinoma of the prostate is seen extremely rare. The aim of this presentation is to report of the patient with coincidental Non-Hodgkin's lymphoma and prostate cancer.

CASE REPORT

A 69-years-old man was referred for urological investigation due to an elevated serum PSA level of 9.10 ng/ml. Digital rectal examination revealed no pathological papable changes except for enlargement of prostate (prostate volume 130 cc). Transrectal ultrasonography guided 8 core prostate biopsy was performed. Histological examination revealed an adenocarcinoma of the right lobe of the prostate with a Gleason score of 2 in one out of 8 specimens.

Treatment options were discussed; the patient chose radical treatment and was admitted to the urology department for radical retropubic prostatectomy.
Medical history included coronary heart disease, primary arterial hypertension, bilateral glaucoma and glucose tolerance disorder. No clinically significant deviations were discovered in pre-operative tests. Radical retropubic prostatectomy was done.
Histological examination confirmed adenocarcinoma of the right lobe of prostate, Gleason 2+3=5. Fig.1. No cancer was detected in the left lobe of the prostate, periprostatic tissues, seminal vesicles, resection margins and obturator lymph nodes. Due to widespread infiltration of lymphocyte-like cells in the right lobe of the prostate an immunohistochemistry was performed and low-grade B-cell non-Hodgkin's lymphoma was discovered. Fig.2. The lymphomatous infiltrate was closely, although not completely, co-localized with the adenocarcinoma areas.

A 3 month follow-up after surgery revealed no pathological changes in CT scans of the lungs, the abdomen, or the pelvic cavity and the PSA value was less than 0.003 ng/ml.

DISCUSSION

Lymphoma of the prostate can be classified into two groups – primary (located predominantly in prostate with or without extension to adjacent tissue with absence of lymph node involvement and a lymphoma free interval of 1 month or greater) and secondary prostatic lymphoma (1,2,3). The incidental finding of a non-Hodgkin's lymphoma following radical retropubic prostatectomy is 0.2% (5). However, primary prostatic lymphoma represents only 0.1% of all non-Hodgkin's lymphoma cases and 0.09% of all prostate neoplasias (1,7)]. Both non-Hodgkin's lymphoma and prostate cancer present likely in elderly patients. Typically, the PSA level in prostatic lymphoma cases is not elevated, however, there are reported PSA-positive non-Hodgkin's lymphoma cases, when PSA level could be significantly elevated, suggesting metastatic prostate cancer (4,6). Typical symptoms for lymphoma of the prostate could be signs of lower urinary obstruction and possible abnormal findings on digital rectal examination (1, 3). On radiological examination, pelvic lymphadenopathy could be observed (1,6).

CONCLUSION

The incidence of primary Non-Hodgkin's lymphoma of the prostate is less than 0.1% (1,7). A case when primary lymphoma of the prostate is combined with the prostate adenocarcinoma is even more uncommon. There are no clear data about the incidence of such cases. The treatment option for localized prostate cancer

is radical prostatectomy. In our case, radical retropubic prostatectomy could be considered the radical treatment also for the Non-Hodgkin's lymphoma, while it did not expand beyond the prostate and additional imaging studies did not show any regional lymph node involvement. However, close follow up of the patient should still be maintained.

Conflict of interest: None

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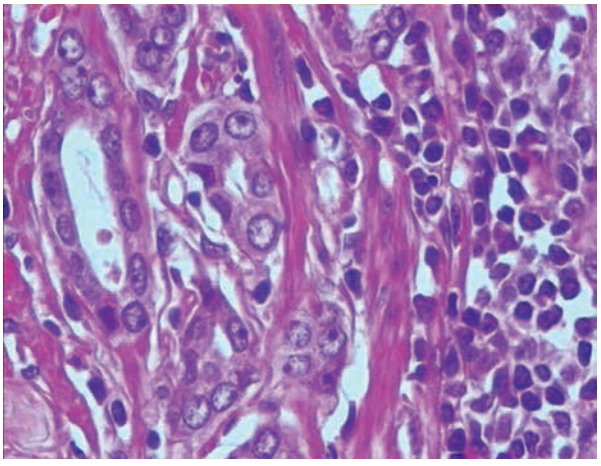

Fig. 1. Acinar adenocarcinoma of the prostate, Gleason 3. Basal cells are lost within the complexes of adenocarcinoma. Markedly enlarged nucleoli are present in cancer cells. Infiltration of immature lymphoid cells is present adjacent to cancer complexes. Haematoxylin-eosin stain, original magnification 400x.

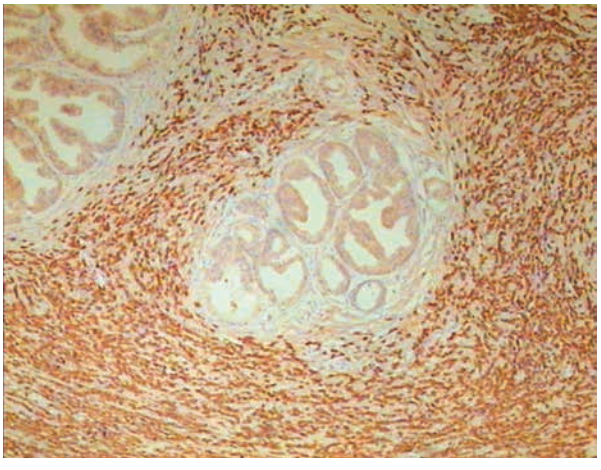

Fig. 2. Infiltration of low grade B-cell non-Hodgkin's lymphoma within the prostate tissues. Immunoperoxidase, anti-CD20. Original magnification 50x.

CASE REPORT

A Case Report of Complicated Multiple Facial Basal Cell Carcinoma Treatment in a Young Woman

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****RECUH Latvian Oncology centre, Riga, Latvia**

Summary
A 36-year-old woman was consulted in Riga Eastern Clinical University Hospital Latvian Oncology centre Outpatient Department with multiple histology-proven basal cell carcinomas. The patient was treated with diode laser 980 nm and immunotherapy and had 5 years disease free survival.
Key words: multiple facial basal cell carcinoma, treatment, laser, immunotherapy.

AIM OF THE DEMONSTRATION

Basal cell carcinoma (BCC) is the most common skin cancer over the world. BCC is a non-melanocytic skin cancer with slow growth tendency and low mortality. BCC typically appears on sun-exposed skin (face, neck). High relapse risk (until 40%) is common for BCC. However, there is no common opinion on the most efficient method for BCC treatment – method, which would ensure the highest possibility of non-relapse and good cosmetic result. In order to evaluate the most appropriate method for BCC treatment longitudinal 5-year follow-up period is required.

CASE REPORT

36-year old patient was consulted in Latvian Oncology centre Riga Eastern Clinical University Hospital with multiple (8) tumors on face skin (5 on cheek and 3 on chin) (Fig.1). Punch biopsy of tumours was performed and morphological diagnosis of basal cell carcinoma with adnexal differentiation (trichobazalioma) was recognised.

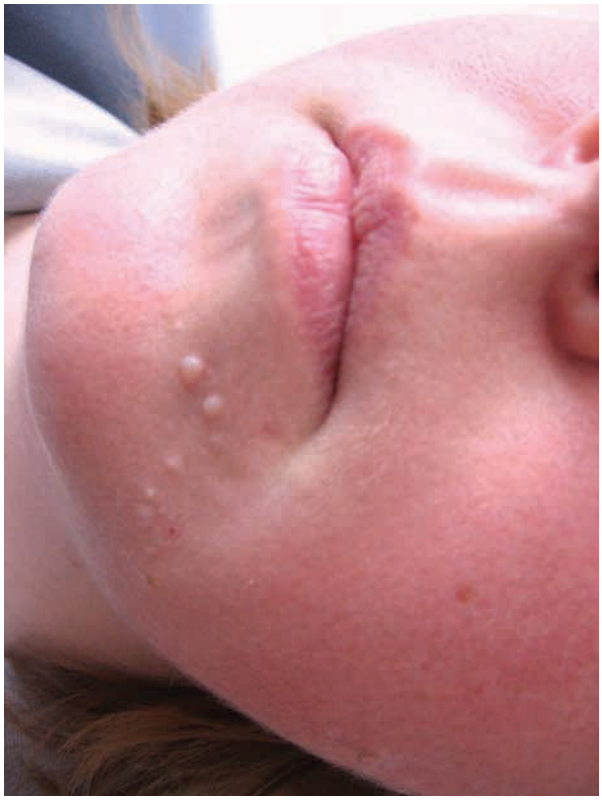

Fig. 1. Five small BCC tumors on the chin
After consultation with maxillo-facial surgeon on February 22, 2006 second consultation was done by oncologist - laser surgeon due to high sensitivity to expected cosmetic result. To remove all lesions two level laser surgery was suggested: level I – for 5 tumours on cheek with a tumour diameter 2-3 mm, level II – for 3 tumours on chin up to 4 mm diameter. On March 8, 2006 laser surgery on left cheek was performed. (Fig.2).

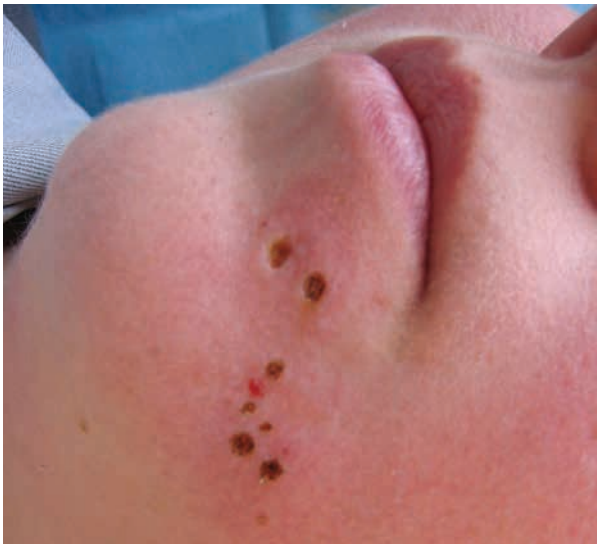

Fig. 2. State after laser surgery
A cytological analysis of tumours showed basal cell carcinoma with inflammation and hyperkeratosis. It was consistent with morphological report. (Fig.3).

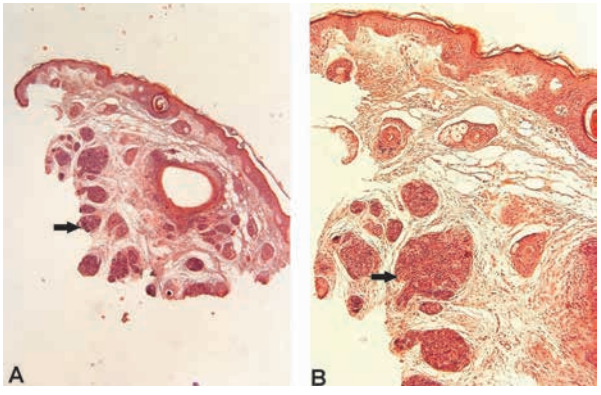

Fig. 3. Basal cell carcinoma with adnexal differentiation. A - The overall view shows the presence of adnexal differentiation including basaloid buds and prominent trichelemmal and follicular differentiation (black arrow). B - High power view showing groups of atypical basaloid with peripheral palisading cells (black arrow). A- magnification×40; B- magnification×200 Hematoxylin-eosin.
On March 22, 2006 second level laser surgery was completed for 3 tumours on chin. After scar-healing (Fig.4) immunologist consultation was allocated. On May 8, 2006 cellular immune parameters were determined and immune therapy was assigned.

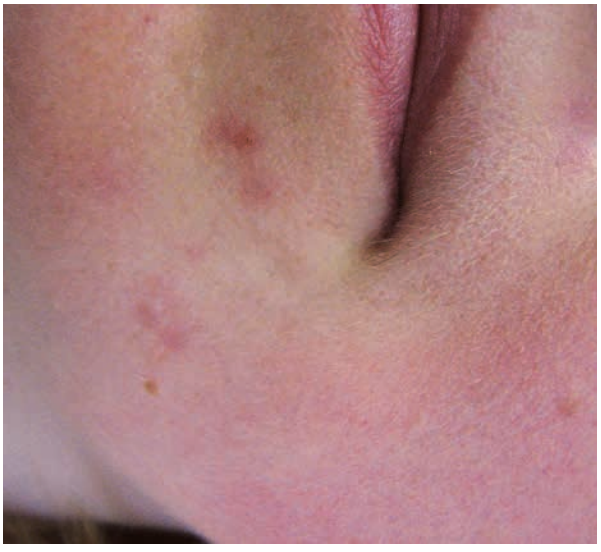

Fig. 4. Clinical outcome 1 month after laser surgery
From November 2006 until December 2010 patient received eight courses of immunomodulation with interferon (IFN) inductor and proteolytic enzymes. The number of CD16+NK cells at the beginning of the therapy had decreased and there were no signs of T system deficiency.
Immunomonitoring was performed during the therapy; cellular immune response was evaluated by measuring CD3+, CD4+, CD8+, CD16+, CD19+, CD38+ and CD95+ cells.
Absolute count of T cell subsets was relatively stable during the therapy. Number of CD16+ cells and activated lymphocytes were slightly increased. During the last control visit on December 27, 2010 no signs of relapse were seen in all 8 places. Also there were no signs of any other suspicious new skin lesion.

DISCUSSION

BCC is a common form of skin cancer, however, quite rare in young age (1, 5). Highlighted case demonstrates complication of diagnostics – the discrepancy between clinical (viral infection) and histological diagnostics (BCC) – as well as difficulty to choose correct treatment method. The age of patient (36 years) and multiple tumours (8) on face forced to search for two-fold method – the most reliable way of treatment (without recurrence) and also the most suitable from the esthetical point of view. Traditionally, surgery, radiation therapy and cryosurgery are used for BCC treatment (2, 4, 5, 6). However, all these methods can lead to rather unsatisfactory cosmetic result in case of multiple tumours, especially located on face. Therefore, laser surgery method was chosen to minimize complications (6). Laser surgery was performed ambulatory under local anaesthesia without any local complications. In addition, the postoperative period was without complications. The patient was able to work and the quality of life was not decreased. All postoperative scars were normothrophic. Immune therapy was assigned to ensure lower risk of new BCC occurrence.

Presented patient with multiple basal cell carcinomas had insufficiency of non-specific effector phase of cellular immunity, which leads to the deviation in antitumor immune response.

IFN plays an important role in tumour microenvironment. Induction of cytokine in the innate and adaptive immune cells could help to overcome tumour escape from immunological control. Production of IFN-alpha is able to enhance Th-1 mediated cellular antiviral and antitumor immunity (8). Various studies demonstrated clinical efficacy of cytokines and other immune response modifiers in the treatment of basal cell carcinoma (3,7). To reach remarkable cosmetic result for multiple BCC with a 5-year disease free survival a treatment should be enabled by multidisciplinary team.

Conflict of interest: None

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CASE REPORT

Liver Damage after Breast Plastic Surgery – Clinical Case Report

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Summary
Since silicone implants were introduced in the early 1960s, those have been widely used for cosmetic and reconstructive breast surgery. Although a recent review has shown no relationship between the silicone breast implant and systemic complications, leakage of the silicone into the tissues and migration to the regional lymph nodes remains a clinical problem. This was the first case in our practice when possibly breast implant material was found in the liver tissue.
Key words: liver, breast implants, complications.

AIM OF THE DEMONSTRATION

To demonstrate relatively rare complication after breast plastic surgery.

CASE REPORT

38 years old women presents with discomfort in the upper right quadrant and epigastric region. Complaints last for approximately 5 years, periodically. The patient reports no itching, bleeding, jaundice, nausea or other dyspeptic complaint. Laboratory tests within normal ranges, except mild anemia.

16 years ago patient had plastic operation for breast enlargement. The mammary prosthesis consisted of polyurethane cover and filled with synthetic thermostable rubber of low molecular structure. There were episodes of allergic reactions with face edema, Quincke's edema and lymphadenopathy starting about 9 years ago. Lymph node biopsy: infiltration of macrophages and atrophy of lymph node. Breast implants were removed 8 years ago. After that there was an episode of hepatosplenomegaly and enlarged lymph nodes 5 years ago.

Ultrasound examination: Right lobe of liver slightly enlarged with multiple hypoechogenic focuses 3-5mm diameter, Fig.1.

It was decided to perform liver biopsy.

Liver biopsy: accumulation of unknown material (fat, air, lypophile radiopaque) in the portal tracts without significant damage of liver parenchyma. Spear-shaped holesterol or fatty acid crystals are not found, there is no damage of hepatocytes or Kupffer cells. There are no data of inflammatory or neoplastic process, fig. 2., 3.

Conclusion: It is possible that material of breast implant has been spread into organism tissues including lymph nodes and liver.

DISCUSSION

According to literature data there are data of breast implants material spread into lymphatic system and other tissues.

Hydrophilic polyacrylamide gel is a nonresorbable soft tissue filler that has been used as implant material for breast augmentation in some countries, particularly from the Asian continent. Many complications associated with hydrogel use have been reported in the clinical literature including inflammation, persistent mastodynia, formation of multiple lumps, poor cosmetic results, glandular atrophy, and significant spread of hydrogel into the surrounding tissue. Data on long-term toxicity is currently unavailable [1]. Since the silicone implant was introduced in the early 1960s, it has been widely used for cosmetic and reconstructive breast surgery. Although a recent review has shown no relationship between the silicone breast implant and systemic complications, leakage of the silicone into the tissues and migration to the regional lymph nodes remains a clinical problem [2]. Through magnetic resonance spectroscopy and atomic emission spectroscopy, silicon compounds were found in the blood of some women with silicone breast implants; silicone and silica have also been found in liver [3].

Infectology Center of Latvia is specialized into liver diseases diferential diagnosis and management. We perform almost one thousand liver biopsies each year, mainly due to chronic viral hepatitis C, but also to diagnose other hepatic diseases, like primary biliary cirrhosis, steatohepatitis, focal liver lesion differentiation and other. This was the first case when possibly breast implant material was found in liver tissue. This case demonstrates very rare and relatively late complication of breast plastic surgery. There is still an open question – what will be the next step? How this material will affect liver tissue in a long term perspective, taking into account that there is a foreign body for many years in the liver and other tissues?

Conflict of interest: None

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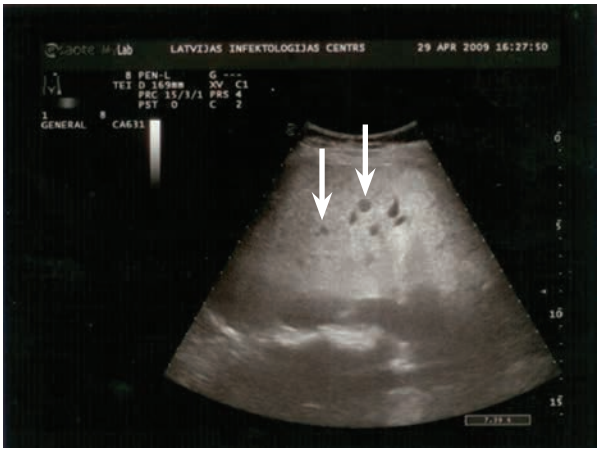

Fig. 1. Right lobe of the liver with hypoechogenic focuses, ultrasound.

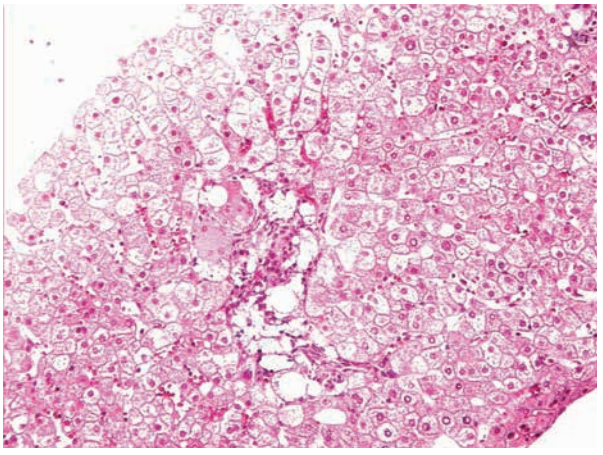

Fig. 2. Liver *core* biopsy: magnification 100 x (H & E). Dilatation of portal tracts due to foam cells. Foam cells are filled with an empty vacuolas. Morphological structure of hepatocytes is not damaged.

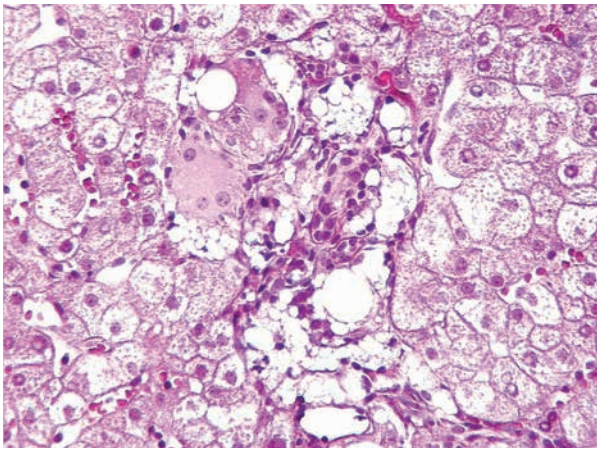

Fig. 3. Liver *core* biopsy: magnification 400 x (H & E).

CASE REPORT

First Liver Transplantation in Latvia for Patient with Primary Sclerosing Cholangitis

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Summary
This is the case of first orthotopic liver transplantation (LT) in Latvia. LT was done due to liver cirrhosis caused by primary sclerosing cholangitis (PSC). After LT patient had two complications – stenosis of portal anastamosis and necrosis of common bile duct (CBD), which were successfully solved with stenting of v.porta and reconstructive operation of CBD.
Key words: liver transplantation, primary sclerosing cholangitis, stenosis of *v.porta.*

AIM OF THE DEMONSTRATION

LT nowadays is a well accepted treatment method for end-stage liver disease and acute liver failure. It is also one of the most expensive treatments. Indications for LT have increased over the last few years and according to European Liver Transplant Registry - 93634 LT (7) were performed in Europe till 2010. Until now Latvia was the last country in Europe, where LT was not performed (2,4). The aim is to demonstrate the first successful orthotopic LT in Latvia as well as management of postoperative complications.

CASE REPORT

A 46-year-old male with diagnosis of PSC, secondary biliary cirrhosis, mechanical jaundice and unspecific ulcerous colitis was admitted to LT waiting list in January 2010. Since 2003 history of PSC is known and during this period the patient was treated in Gastroenterology center of Pauls Stradins Clinical university hospital several times. Due to mechanical jaundice, stents were put in the bile ducts. Because of suspicious for malignant bile duct disease in 2003 biopsy of the liver was performed and cirrhosis of the liver was proved. In 2007 unspecific ulcerous colitis was diagnosed, which currently is in remission.

Operation was performed in January 13, 2011. The donor was 62-year-old male, who had spontaneous hemorrhage in brain with determined cerebral death. The recipient was admitted to hospital in late evening with mechanical jaundice, ascites, encephalopathy and intoxication. Screening analysis were performed and common bilirubin level was found to be extremely elevated - 171 mkmol/l and serum albumin decreased - 11g/l. Operation team consisted of four general surgeons and two vascular surgeons, who helped with donor liver preparation procedure and vascular anastomosis during LT. *Piggy-back* modification of LT was performed with three vascular anastomosis and CBD anastomosis with jejunum loop. Explantation of recipient's liver was technically sophisticated due to multiple adhesions in abdominal cavity after previous operation. It resulted in great amount of blood loss during operation. In spite of applied Cell-Saver system ten liters of blood specimens were transfused. Operation time was ten hours and 45 minutes, donor liver ischemic time was nine hours and v.portae occlusion time one hour. After operation patient was admitted to intensive care unit and extubated in late afternoon. Immunosuppressive therapy with Cell-Cept, Advograf and Prednisolone was started already during operation. In the first five postoperative days patient felt quite good and was active with little complains of flatulence. During first 24 hours 1000ml of serous liquid from abdominal cavity drainage was collected. On day six postoperatively, patient had pain in abdomen, flatulence, signs of intoxication and leukocytosis. Ultrasound (US) and computer tomography (CT) angiography of abdomen were performed and 70% stenosis of v.portae anastomosis was detected (Fig. 1). In the first stage dilatation with balloon angioplasty through hepatic vein by transcutaneous approach was performed. Initially it gave good result, but in the next morning the same complains occurred. In January 20, 2011 transcutaneous stenting of v.portae was performed (Fig. 2). Two days later patient was transferred to surgery department and discharged from hospital in good shape on the 21st day after operation. Patient came for weekly check-ups. On February 10 patient started to complain of formation in right under rib region. Bilioma in sub-hepatic space was found and drained by transcutaneous drain under US guidance. In five days it resolved and drainage was removed, but patient still had discomfort in abdominal cavity and mild leukocytosis. Abdominal MRI showed dilated intrahepatic bile ducts with cholestasis and possible necrosis of CBD (Fig. 3). On February 24, 2011 reoperation was performed and destructive CBD discovered. It was resected and anastomosis between liver and jejunum loop on stent drainage was established. Postoperative period was without complications, operation wound healing was good and patient was discharged from hospital in the 7-th postoperative day. The last control was 3 months after operation – patient was in good condition, analysis normal and he continues immunosuppressive therapy.

DISCUSSION

As LT is very complicated operation, different complications occur quite often. They can be obstructive and non-obstructive. Causes of obstructive complications are related to problems with hepatic artery, bile ducts, portal vein and inferior vena cava. Non-obstructive complications include allograft non-function, rejection, viral infection and others. In our case we had two obstructive complications – stenosis of portal vein anastomosis and ischemic necrosis of CBD (6).

The incidence of portal venous complications following LT is considered to be relatively uncommon in comparison with hepatic arterial complications, but they can lead to graft loss. In literature three ways are mentioned to solve this complication – balloon angioplasty, percutaneous trans-hepatic portal venoplasty (PTPVS) with stenting or surgical treatment (thrombectomy, anastomosis revision or re-transplantation). We did not choose surgical management of this complication, because of possible technical difficulties due to postsurgical fibrosis and limitations in the length of the involved venous structures. It was right solution, because PTPVS was successful and during re-laparotomy we found adhesions and fibrosis in sub-hepatic region. The first step for solving portal vein stenosis was balloon angioplasty, which gave good early result in our case, but as described in literature recurrence rate is relatively high (28,6-36,8%) (3). Second reason why immediate PTPVS was not performed is possible stent thrombosis, because of slow venous blood flow. As balloon angioplasty gave recurrent stenosis, PTPVS with 14mm diameter, 40mm long stent was performed and it was successful with no recurrent stenosis. For prophylaxis of venous thrombosis patient received Plavix for two months and now continues with ThromboASS 100mg daily.

Regarding to complication with CBD – surgical operation with reconstruction of anastomosis is the only way to solve this problem. The incidence of biliary leakage according to literature is 9.5% (1) and in our case, the reason can be ischemic CBD of graft. As the patient had CBD anastomosis with jejunum loop, endoscopic management of bile duct problems was technically impossible and formation of new anastomosis was the method of choice.

Conflict of interest: None

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First Liver Transplantation in Latvia for Patient with Primary Sclerosing Cholangitis

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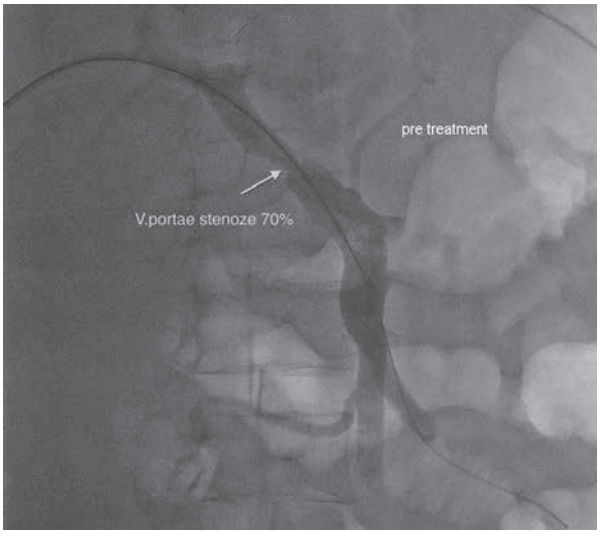

Fig. 1. Stenosis of venae portae

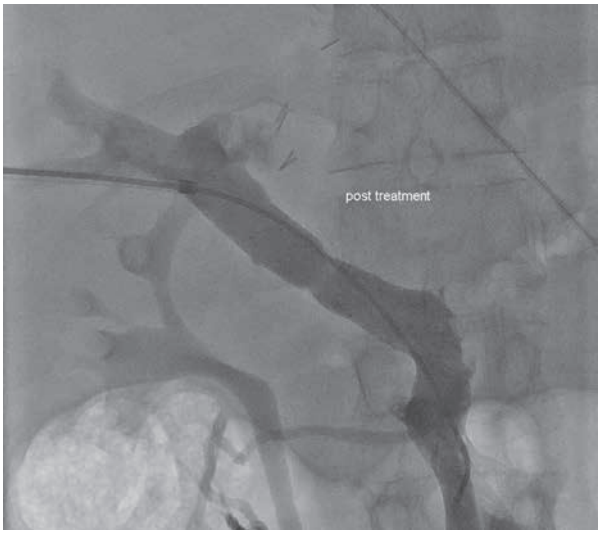

Fig. 2. Venae portae after stenting

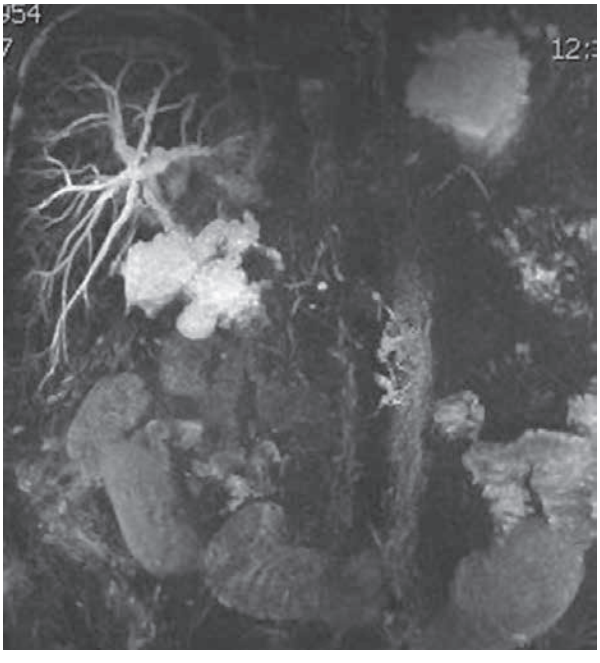

Fig.3. Upper abdominal MRI

CASE REPORT

The Treatment of Acetabular Fracture Complications in a Combination of Post-traumatic Hip Joint Osteoarthritis and Femoral Fracture for Polytrauma Patient

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Summary
Pelvic bone fractures are related to a high energy injury. Therefore with an increase of the traffic intensity simultaneously grows the number of polytrauma patients, who have pelvic bone fractures of various severity levels. In the case of acetabular fractures, as the most frequent complication is the hip joint post-traumatic degeneratively destructive osteoarthritis, in whose treatment very often the hip joint arthroplasty is necessary. The frequency of this complication is affected by the precision of repositioning and the strength of fixation of an acetabular fracture.
Key words: polytrauma, acetabular fracture, ilioinguinal and anterior retroperitoneal approach, hip joint arthoplasty.

AIM OF THE DEMONSTRATION

The aim of this article is to demonstrate a case of successful repair of hip joint post-traumatic osteoarthritis in a case of a polytrauma patient (ISS-34) combined with an ipsilateral femoral fracture. And also to demonstrate that in this case the retroperitoneal approach would have given a possibly better postoperative result in comparison with the ilioinguinal approach and avoidance of hip joint arthoplasty.

CASE REPORT

A 28-year old female patient was hospitalised after a road traffic accident, in which she suffered as a car passenger. After examination a diagnosis was made: polytrauma. Brain contusion. Bilateral pulmonary contusion. Splenic rupture. Liver rupture. Comminuted and open distal metaphyseal fractures of the left forearm and left upper arm. Open diaphyseal left femoral fracture with a bone defect. Trancetabular fracture and dislocation of the left side pelvic bone both columns and hip joint contortion. Extensive torn wound with a soft tissue defect in the gluteal area. Due to splenic and liver rupture, an undeferrable laparatomy, splenic removal and liver suture are performed, as well as pleural cavity drainage, hip joint reposition, wound treatment and the fixation of the fractured limbs and the pelvic bone with external fixation apparatus.

Due to the severe condition and extensive secondary healing wounds, open reduction and osteosynthesis are carried out only one month after the injury moment. As an encumbrance to the operation remain continuous intubation with a secondary reciprocal pneumonia, extensive wound and initial pressure sores in the left gluteal area, secondary healing wound in the left femoral fracture area. The femoral bone was fixed with an intramedullar locking nail. At the moment of the operation the front lower retroperitoneal or Stoppa approach was not yet introduced, therefore the pelvic bones were fixed with a plate using the illiolinguinal approach. The combination with the posterior approach was impossible due to extensive wounds in the gluteal area, therefore the back column reposition was incomplete. After the operation the hip joint post-traumatic ostearthritis rapidly progressed on the patient. And six months after osteosynthesis, it was found that the patient had ~40 ° flexion contracture, and the left leg shortening by ~ 3.0 cm. When carrying out the radiological examination (Fig.1), it was noted that there was a coalesced dislocated back column fracture, and hip joint subluxation with a partial penetration of femoral head in the small pelvis.

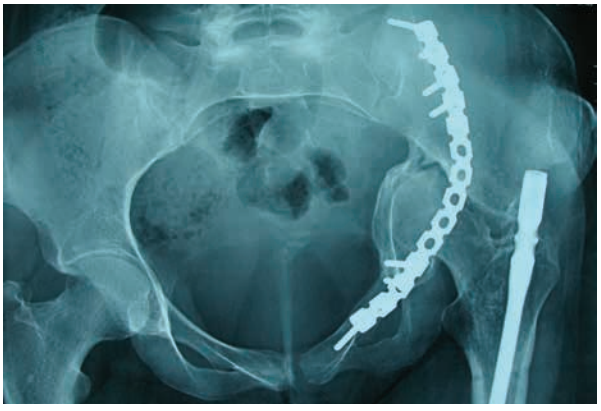

Fig. 1. Plain radiograph of the pelvic bone in six months.

A femoral fracture in six months with weak consolidation that is also affected by the lost bone fragment at the injury moment. The patient moves with a wheelchair, walking with crutches is impossible due to the hip joint flexion contracture. Notable pain syndrome in the left hip joint projection place, outer and inner rotation 0 °. To stimulate the coalescence of femoral fracture, the dynamisation of the fracture is carried out by removing the locking screw.

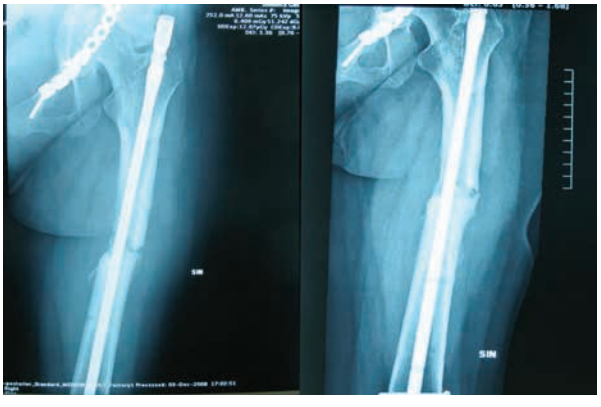

Fig. 2. Plain radiographs of a femoral bone in six and twelve months.

When carrying out next radiological control after 12 months from femoral and pelvic osteosynthesis, a seeming consolidation of femoral fracture is noted (Fig.2.). In recurrent stage operation femoral intramedullar nail was evacuated to prepare the patient for the left hip joint arthoplasty. But after one month in a result of a repeated injury the patient underwent a refracture of the left femur (Fig.3).

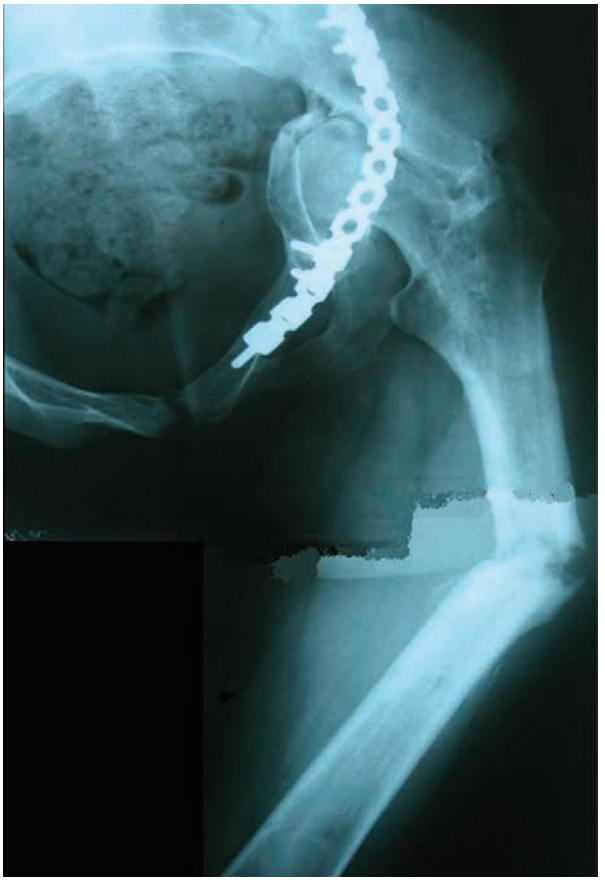

Fig. 3. Plain radiograph shows femoral refracture in one month after removing the intramedullary nail.

In a format of council a decision was made to perform a hip joint plastic with autograft and total arthoplasty with uncemented endoprosthesis and to simultaneously carry out femoral fracture fixation with a revision femoral component. The operation approach – posterior lateral.

After the performed arthoplasty equal leg length was achieved and the motion extent in the left hip joint was almost completely renewed. And in one year after hip replacement we got a good radilogical and clinical outcome (Fig.4).

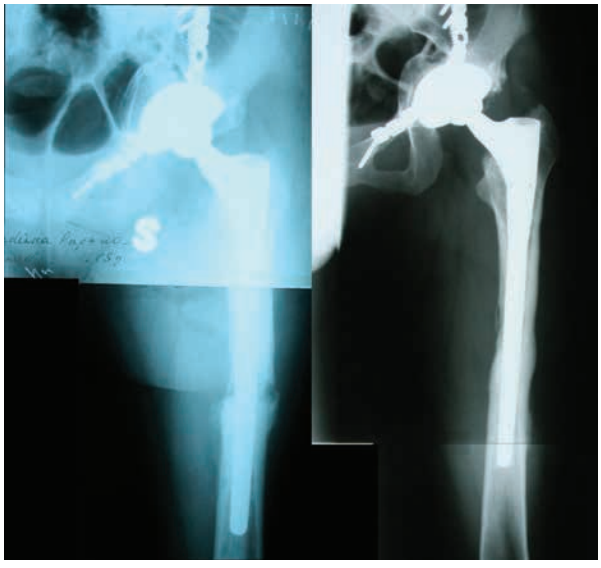

Fig. 4. Plain radiographs three months and one year after hip replacement.

DISCUSSION

There are various surgical approaches used for the treatment of the pelvic bone intra-articular fracture. The most well-known and widely used in practice is the illioinguinal approach. Using this approach the front column of the pelvic bone can be well visualised, but in order to visualise and fix the back column an additional approach from the back side is necessary. On separate clinical cases posterior approach is not possible because of various additional conditions. In the described case those were large wounds in the gluteal areas. In such cases an alternative is modified Stoppa approach (1.2), because with this approach it is possible to visualise well not only the anterior column, but also to reduce and fix posterior column (3.4). In such a way avoiding using additional posterior approaches.

Post-traumatic arthritis may develop after an injury, if the joint in which the bone and cartilage do not heal properly, is no longer smooth. As a result this leads to excessive wear on the joint surface and the development of osteoarthritis. A serious joint fracture or torn ligament can also lead to post-traumatic arthritis. The joint can be made unstable by any injury to the ligament, supporting muscles or joint. Eventually arthritis may develop from the increased stress on the joint cartilage.

Posttraumatic hip joint osteoarthritis in combination with femoral bone fractures is rarely noted. Femoral fracture or repeated fracture is fostered by limitations of the hip joint motion even by a small trauma or clumsy movements. In such cases the standard tactics is to firstly to coalesce the femoral fracture and only at the second stage the hip joint arthoplasty is performed. Simultaneous hip joint arthoplasty and femoral osteosynthesis can be chosen as a method, if there is pronounced hip joint contracture due to which it is not possible to walk with supporting means.

Conflict of interest: None

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CASE REPORT

Both Knee Re-revision Operations with Different Types of Endoprosthesis after Septic Complications

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Summary
We report about the patient who underwent seven replacement operations in both knees during twelve years period. Different types of implants were used due to clinical situation and septic complications.
Key words: knee joint osteoarthritis, total knee joint replacement, re-revision, septic loosening, rotating-hinge.

AIM OF THE DEMONSTRATION

The aim of the article is to demonstrate severe knee joint osteoarthritis patient's treatment possibilities with different types of knee endoprotheses.

CASE REPORT

Seventy-six years old male patient with severe osteoarthritis suffered from pain in both knees from 1986. In 1990 pain in the right knee became permanent. Patient complained about limping and increasing deformity. Preoperative treatment included nonsteroidal antiinflammatory drugs, physiotherapy, intraarticular injections of dexamethason. When conservative treatment became non-effective we decided to perform total knee replacement (TKR) of right knee joint. During first TKR in November 21, 1995 we applied posterior cruciate ligament retaining knee resurfacing prosthesis (SKI Waldemar Link GmbH Hamburg) (Fig. Nr.1). 14 months later (January 21, 1997) patient underwent TKR of the left knee joint with cruciate ligaments sacrificing knee resurfacing prosthesis (SKI Waldemar Link GmbH Hamburg) due to clinical signs of the damage of both cruciate ligaments (Fig. Nr.2). Both endoprothesis were fixed with bone cement.

In post operative period patient was free of pain and his activity level was pretty high (patient took part in a different kind of sport activities like volleyball and swimming).

In the beginning of 2001 patient started to complain about pain, swelling and also some limitation of movements in the left knee. Radiographs showed asymmetry of joint space and radiolucent line between the implant and bone (Fig. Nr.3). 4 years and 10 month after primary TKR of left knee revision operation was performed (Fig. Nr.4). To gain stable and painless knee we used femoral and tibial components of revision endoprosthesis with prolonged polished stems and joint surfaces with stabilized tibial insert with posterior peg (Johnson&Johnson TC3). The bone defects were filled with bone allografts. We used bone cement with antibiotic (Gentamicin) for fixation of femoral and tibial metaphyseal parts. There was found Staphylococcus xylosus in the microbiological samples taken during operation. Despite that the wound healed primarily. Postoperatively the patient got parenteral antibiotics for 3 weeks and then oral antibiotics for 1 month.

From 2004 pain and swelling appeared in the right knee. 8 years and 2 months after primary operation, the revision TKR was performed with similar implants as in the left knee (Johnson&Johnson TC3) (Fig. Nr.5,6). The allografting and bone cement with antibiotic (Gentamicin) were used.

Both of removed implants were with massive polyethylene wear on the joint surfaces.

From 2007 patient had complaints about pain, swelling and limitation of movements in the left knee again, but no microbes were found in the joint fluid sample before the operation.

The re-revision operation of left knee joint was done with rotating-hinge endoprosthesis (Waldemar Link company´s Endo-model) (Fig. Nr.7,8) 11 years after primary TKR. Both femoral and tibial components (including stems) of endoprosthesis were fixed with antibiotic (Gentamicin) loaded bone cement. During operation no microbes from tissue samples were found. (Fig. Nr.11,12). Half a year after last operation patient had no pain and satisfactory range of movements (flexion 95 degrees, full extension) in both knees. Patient could walk without walking aids and any limp.

DISCUSSION

TKR have had excellent results, with multiple studies showing survival rates greater than 90% at follow-up times of 10 to 20 years.[1-6] Development of the knee replacement operations with different types of endoprosthesis still continues. Numerous prostheses have been developed to improve the durability and function of these procedures. However, there has been controversy regarding whether the posterior cruciate ligament (PCL) should be retained or removed during procedure.[8]

The main cause for primary implant failure was the wear of polyethylene surface. (Fig. Nr.9.10.). About twenty years ago one of the philosophies was to use cruciate ligaments retaining knee resurfacing prosthesis. We also used this type of prosthesis in the right knee of our patient for primary TKR. This concept is till now very popular for young and high demanding patients[7], however the risk of wear is higher than in cruciate ligaments sacrificing endoprosthesis due to not perfect balanced ligaments. The primary operation of our patient was performed when sophisticated instruments for the alignment of prosthesis components were not available. Now the further development of instruments gives us possibility to avoid alignment failures. The quality of materials for joint surfaces have also improved like in vacuum sterilised polyethylene what we used later.
During the second revision of left knee the PCL sacrificing endoprosthesis was used. The concept of knee joint revision with prolonged polished femoral and tibial stems is very popular, but our experience shows also good results with fully cemented long stem implants like rotating-hinge enoprosthesis (Endo-model). This type of prosthesis has advantages for difficult revision operations and re-revision cases with collateral ligament insufficiency and bone loss especially after septic cases.

Conflict of interest: None

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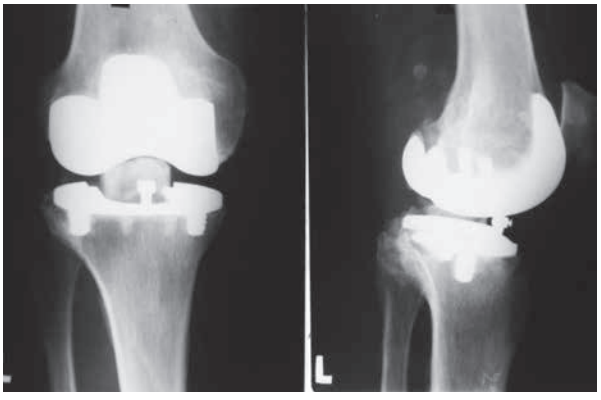

Fig 1. Primary TKR in right knee due to knee joint osteoarthritis (1995) with PCL retaining endoprosthesis.

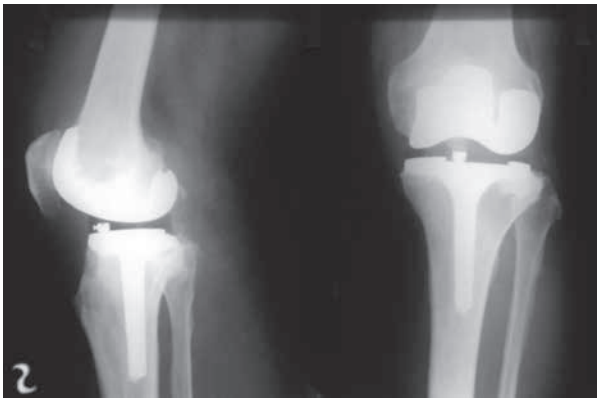

Fig 2. Primary TKR in left knee due to knee joint osteoarthritis (1997) with PCL sacrificing endoprosthesis.

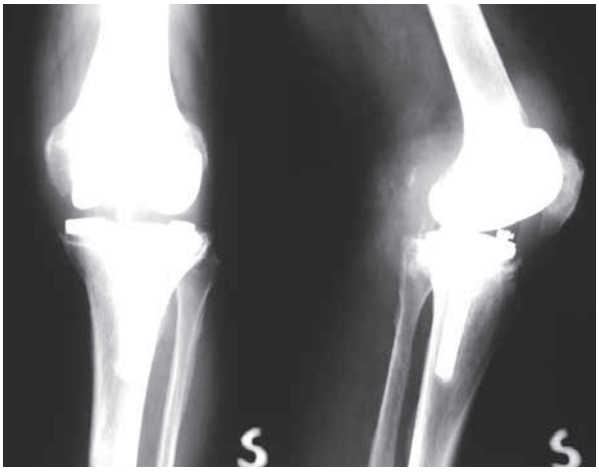

Fig 3. Septic loosening of both components in left knee 4 years and ten months after primary TKR.

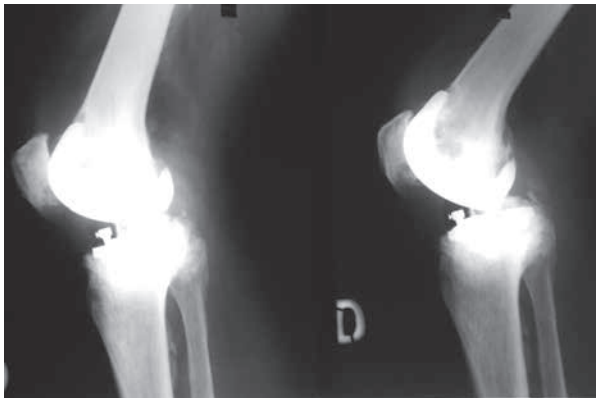

Fig 5. Aseptic loosening of both right knee prosthesis components 8 years after operation.

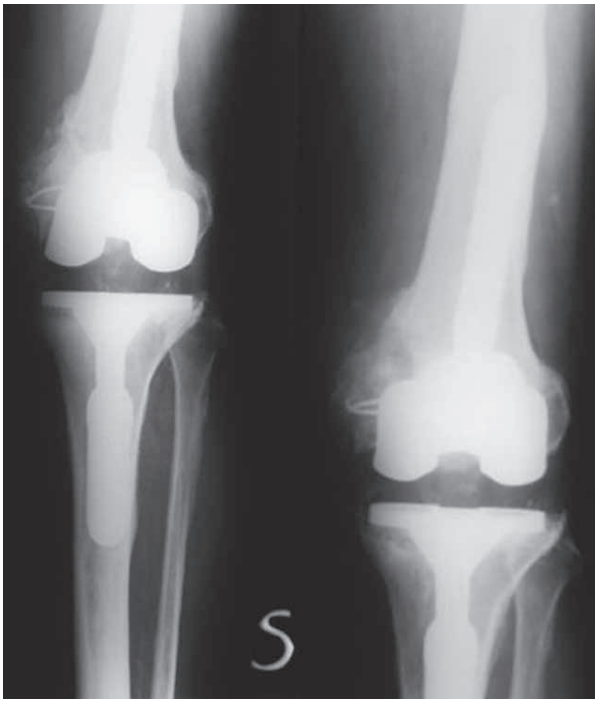

Fig 4. In 27.11.2001 revision TKR (J&J) in left knee due to septic loosening of both components.

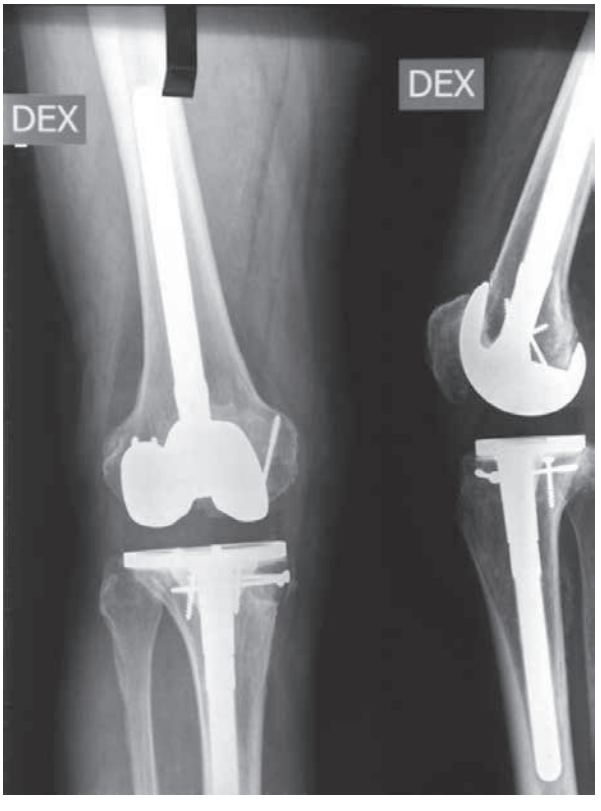

Fig 6. In 09.02.2004 revision TKR (J&J) in right knee with long stemmed endoprosthesis.

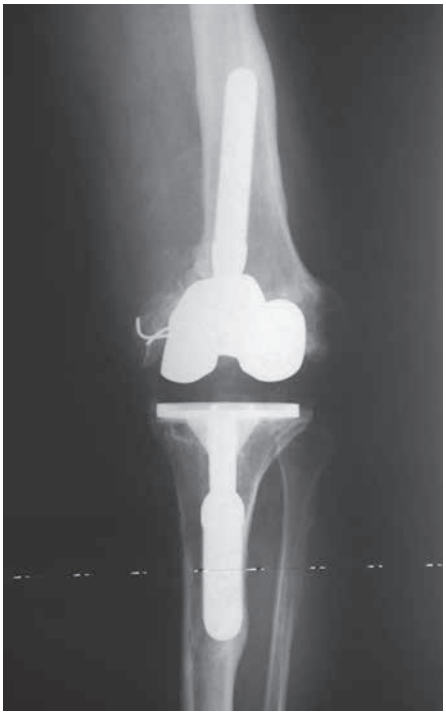

Fig. 7. Septic loosening 6 years after the first revision in left knee.

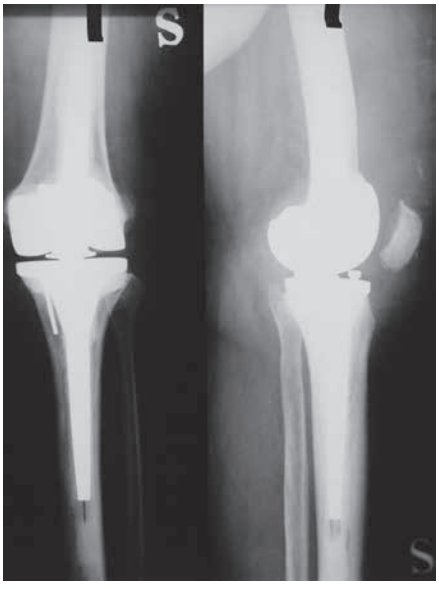

Fig 8. In 09.01.2008 re-revision´s second step - TKR by rotating-hinge endoprosthesis.

Fig 9. Removed endoprosthesis due to polyethilene wear.

Fig 10. Removed endoprosthesis due to polyethilene wear.

Fig 11. Flexion (105°) of both knees after last re-revision.

Fig 12. Full extension of both knees after last re-revision.

CASE REPORT

Human Dirofilariasis in Latvia – the First Case in Surgical Practice

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Summary
Though dirofilariasis in humans occurs rarely, the number of cases due to *Dirofilaria repens* has been increasing worldwide over the last decade. Climatic changes and animal migration extend geographic area of human dirofilariasis, now including Latvia. The diagnosis is based on histopathological features. Surgical excision is the only curative treatment. Practitioners should bear in mind the possibility of human dirofilariasis in residents of Latvia.
Key words: human dirofilariosis, *Dirofilaria repens*, surgical disease, out-patient surgery.

AIM OF THE DEMONSTRATION

Aim of the demonstrationis to show a case of previously unknown surgical disease in residents of Latvia that can be encountered in out-patient surgery.

CASE REPORT

The patient, 32-years-old woman, attended surgeon with complaints about continuous subfebrile temperature and discomfort in the lower abdomen, where a subcutaneous nodule was found by the patient herself. Her past history revealed no travel outside of Latvia for 4 years. About 9 months before this visit the patient suffered from swelling, redness and induration in her right shank accompanied by febrile temperature during 5 days. After 2 weeks of quiescence new inflammation around her right knee appeared, and then gradually shifted to the right thigh, the right gluteus, reaching the lower abdomen. When the induration in the abdominal wall disappeared, the mass was found.

Clinical examination showed a sensitive pea-sized nodule located in the subcutaneous tissue in the middle of hypogastric region. No redness or other visual changes of the skin were present. Fluctuation was absent as well. After incision, a grey capsule (measuring 0.5x0.7 cm) was revealed deeply in subcutaneous adipose tissue. The capsule ruptured spontaneously during excision and worm structures appeared (Fig.1).

The operation material was submitted for laboratory investigation. At gross examination soft tissue fragments were found measuring 2.2x1x0.5cm. White thread-like structures, measuring up to 5 cm in length and 0.1 cm in diameter were grossly visible as well. The longest thread-like structure was sent for specific evaluation in the parasitology laboratory. Other thread-like fragments measuring up to 2 cm in length were submitted for histology. The tissue material was processed in the tissue processor, embedded in the paraplast, cut into 4 micron thick samples on the slides and stained by haematoxylin-eosin and PAS. Microscopic investigation showed fat tissues with foci of granulations containing parasite fragments (Fig.2). The worm was surrounded by infiltrate composed by epithelioid cells, eosinophils, lymphocytes, plasma cells and macrophages. The transverse section of parasite showed thick multilayered cuticule with longitudinal ridges on external part, well developed longitudinal muscle layer on inner part and the internal organs of parasite (Fig.2). According to the specific morphological outlook *Dirofilaria repens* was diagnosed, which was verified by experts in parasitology. One week after excision the patient had no complaints and the body temperature was within normal range.

DISCUSSION

Human dirofilariasis due to *Dirofilaria repens* is a zoonosis ordinarily affecting the dogs and, more rarely, cats. It is transmitted by *Culicidae* mosquitoes, present only in the Old World. Endemic foci of *D. repens* are located in Southern and Eastern Europe, Asia Minor, Central Asia and Sri Lanka, with the highest prevalence in Italy (Pampiglione *et al.*, 1995). The increasing number of new cases suggest that human dirofilariasis is an emergent zoonosis (Pampiglione *et al.*, 2001). Currently, human dirofilariasis is considered as an emerging disease in the Rostov Region in Southern Russia (Kramer *et al.*, 2007; Kartashev *et al.*, 2011). Two cases of human subcutaneous dirofilariasis are reported in Slovakia (Ondriska *et al.*, 2010).

An increase in the geographical range of dirofilariasis and the risk of human disease may be due to various factors: climatic changes, spreading of canine dirofilariasis, an increase of pet travels, and improvement in the network of pathological services. A growing degree day-based forecast model has been developed to predict the occurrence. The model is based on evidence that there is a threshold of 14 °C below which *Dirofilaria* development will not proceed in mosquitoes, there is a requirement of 130 growing degree-days for larvae to reach infectivity, and there is a maximum life expectancy

of 30 days for a mosquito vector (*Genchi et al.*, 2011).
The age of the patients varied from 4 months to 100 years, with no difference in incidence between sexes (Pampiglione and Rivasi, 2000). The nematodes penetrate the body of the host in the form of infecting larvae by the bite of the mosquito. The larvae migrate through the dermis to the subcutaneous tissue, where they mature either in situ or after migration to other sites. The parasite appears most frequently in the upper half of the body, particularly in the head, ocular region and upper limbs (Pampiglione *et al.*, 2001; Kramer *et al.*, 2007). It is hypothesized, that at the point where they stop, they evoke an acute inflammatory reaction leading to formation granulation tissue that blocks the nematode and causes its death (Pampiglione *et al.*, 2001). The diagnosis usually is based on histopathological features, but immunohistochemical, serological and molecular methods are more sensitive and specific (Pampiglione *et al.*, 2001; Kartashev *et al.*, 2011; Ondriska *et al.*, 2010). As the rate of human dirofilariasis increases in proportion to canine infection (Kartashev *et al.*, 2011), the measures regarding prophylaxis in dogs and control of mosquito populations may decrease the spread of *D. repens*.
Thus, a new entity of surgical pathology has emerged in Latvia. The surgeons and general physicians should be aware of dirofilariasis as it can be encountered in out-patient surgical practice even in residents of Latvia without recent travelling experience. The treatment of dirofilariasis as well as the organization of diagnostics has entered the daily work of surgeon.

Conflict of interest: None

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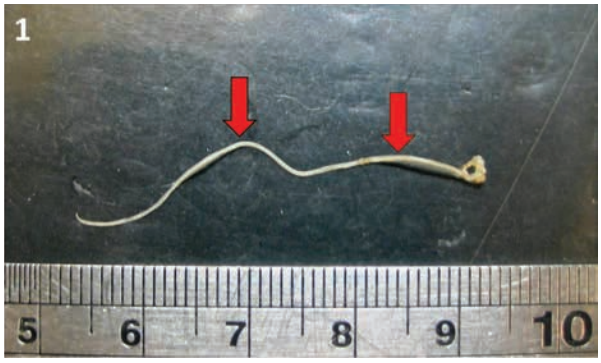

Fig. 1. The removed *Dirofilaria repens* in native specimen. The worm is highlighted by arrows.

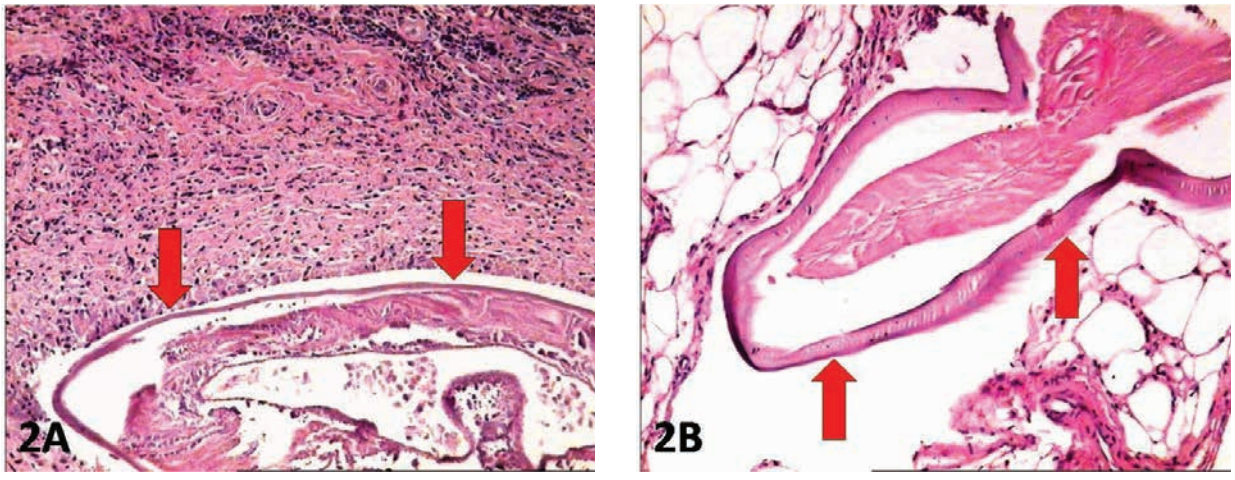

Fig. 2. *Dirofilaria repens* in tissues. Note the granulation tissue in 2A as well as the internal structure of the parasite in 2B. The worm is highlighted by arrows. Haematoxylin-eosin, original magnification 50x (2A) and 100x (2B).

CASE REPORT

Recurrent Pericarditis in a Pediatric Patient

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Summary
Recurrent pericarditis is a chronic condition with the recurrence of pericardial effusion within 3 months after the documented acute pericarditis. The knowledge about this disease is based on observations in adults (6). The common identifiable causes of pericardial effusion in children are a prior cardiac surgery, bacterial pericarditis, malignancy or connective tissue disorders. In a significant number of children, however, despite extensive investigation, it is not possible to identify a clear aetiology. A viral cause is often suspected, though rarely confirmed. There are only few reports on the clinical course of idiopathic pericardial effusions in children. We demonstrate a case of recurrent idiopathic pericarditis with 3 episodes of recurrence within a period of 6 months with no autoimmune disease present, not related to prior surgery or malignancy.
Key words: recurrent pericarditis, paediatrics.

THE AIM OF THE DEMONSTRATION

The aim of the demonstration is to show the case of recurrent idiopathic pericarditis with 3 episodes of recurrence within a period of 6 months with no autoimmune disease present, not related to prior surgery or malignancy.

CASE REPORT

The female patient Z. was first hospitalized at the age of five years on 01.07 .2009. She had a history of the disease lasting two weeks, it had started with nausea which was observed for one day, then the girl had elevated temperature up to 39°C every second day, she became apathetic, slept a lot, complained about pain in cardiac region. The amoxicillin was prescribed by general practitioner and she received it for 10 days with no positive dynamics. No prior history of any serious disease as well as no history of autoimmune or cardiac diseases in the family detected.

She was admitted in poor general condition, breathing with an use of accessory muscles but no crepitation or crackles auscultated, deep-sounding heart sounds, but no murmurs detected, no hepatomegaly present. The thoracic X-ray showed cardiomegaly (the cardio-thoracic ratio was 0,67). Echocardiography revealed anatomically normal heart, LVDd 34mm, fractional shortening (FS) 49%, ejection fraction >60%, circular significant pericardial effusion 12-15mm. Blood count : white blood cells 9.53 $x10^3$, red blood cells $3,7x10^6$, haemoglobin 8.9g/dl, platelets $274x10^3$, C reactive protein (*CRP*) 118,95 mg/dl. Empirical antibacterial treatment with Oxacilline and Ceftriaxone, Furosemide and anti-inflammatory therapy with Ibuprofen was started. Blood cultures were negative. The pericardial drainage was performed and there was 120ml of sero-sangvinous fluid. Cytological examination of the fluid showed sparse neutrophyl leucocytes .After the drainage the fever disappeared. Abdominal ultrasound was without pathological findings. She was consulted by rheumatologist, no elevated auto-antibodies observed (detailed immunological examination performed), and no data about auto-immune disease present. No possible viral cause was detected (enterovirus, adenovirus, cytomegalovirus, Parvo B19, coxaki and hepatitis B viruses excluded), borreliosis excluded. Antibacterial therapy was stopped. On 28.07.09. there was an elevation of temperature up to 38.9°C and the blood count revealed *Micrococcus* therefore she received antibacterial therapy with gentamycine for 7 days. There was maculous rash on the body on 05.08.09. , the girl was consulted by infectologist and allergic reaction suspected and antibiotics discontinued. Since 11.09.09. no elevation of the temperature observed, the echocardiography was without pathological findings, normal blood count and C reactive protein was 8,1 mg/L. The girl was discharged home with outpatient visit scheduled within 2 weeks.

Since 22.09.09. the girl had episodes of elevated temperature up to 38°C and complained about the pain in thoracic cavity. On 24.09.09. blood count was within normal range but C reactive protein 15,5 mg/l. No hepatosplenomegaly was detected. Normal urine analyses. The thoracic x-ray shoved cardio-thoracic ratio (CTR) 0, 6. The echocardiography showed small amount of pericardial effusion up to 5mm and otherwise normal heart. The girl received treatment with Ibuprofen and diuretics, the effusion disappeared within next few days, no temperature reactions observed and she was discharged from the hospital on 28.09.09.

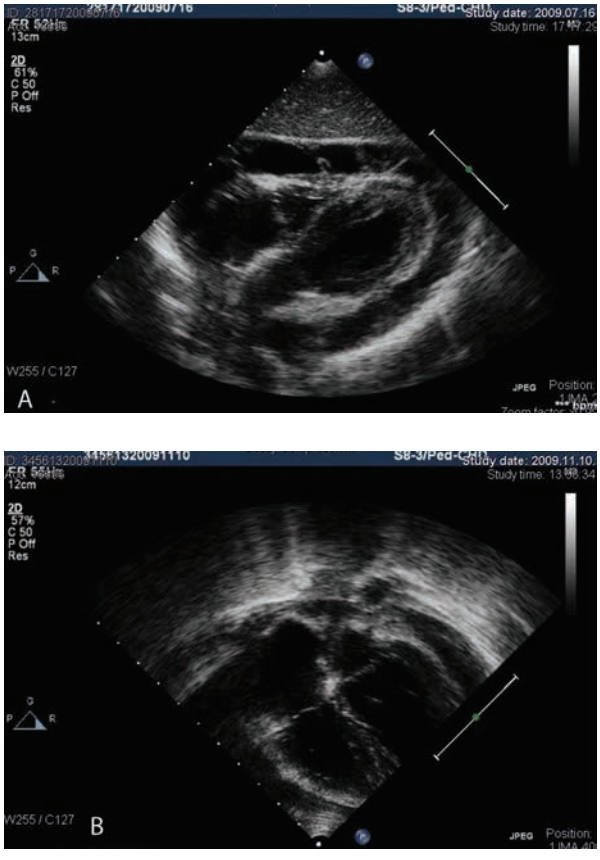
The third time the patient was admitted urgently on 10.11.09. , she had elevated temperature up to 38-39°C since 08.11.09., but tachipnoe since 10.11.09. Thoracic x-ray showed cardiomegaly, CTR 0, 69, left sided pneumonia suspected. Echocardiography revealed fluid collections 8-12mm in pleural spaces bilaterally and circular pericardial effusion up to 13mm

and otherwise anatomically normal heart with normal valvular function. At the admission the treatment with intra venous vancomycine and ceftriaxone, orally Ibuprofen 30mg/ per kg/per day and furosemid started. Blood cultures were negative and antibacterial therapy was discontinued and anti-inflammatory therapy with ibuprofen continued. Mantoux test was negative. Blood immunology showed normal levels of *IgA, IgM, IgG, IgE* was slightly elevated up to 180(normal limits<60), *TNF alfa, C2, C4,* cistatine levels were within normal ranges. There were no auto antibodies: *anti DNS, ENA*, anti cardiolipin antibodies, *p-ANCA, c-ANCA* detected. Serum amiloid A levels were increased up to 983(norm<6.4), *CRP* 66mg/L. The girl was consulted by rheumatologist, the immunological changes were not specific, no data about the connective tissue disease present. On 27.11.09. echocardiography showed no pericardial and pleural effusions and anatomically normal heart. Controlled blood count and *CRP* were within normal ranges. The girl was discharged home on anti-inflammatory treatment which was discontinued after 2 weeks. Afterwards she was followed-up by pediatric cardiologist but no more episodes of recurrence or manifestation of other rheumatologic or oncological diseases observed within next 2 years.

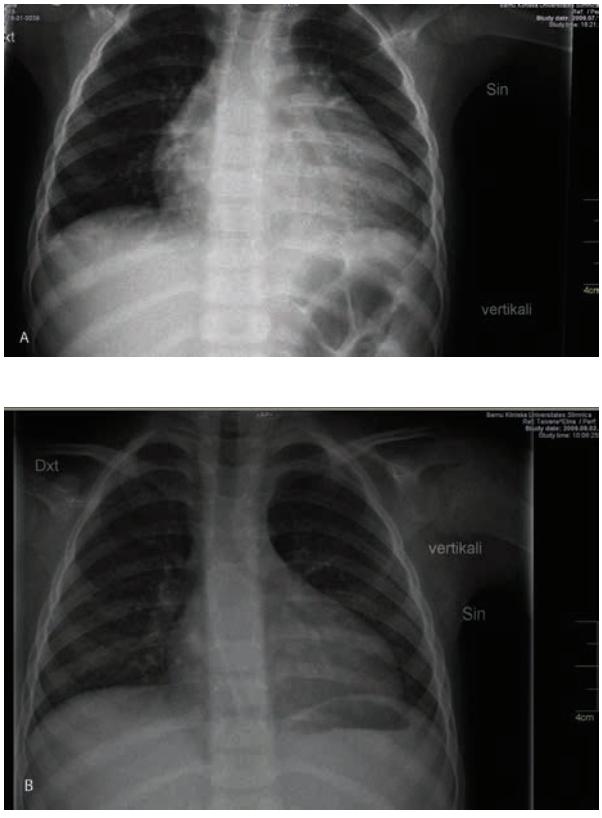
DISCUSSION

Recurrent pericarditis is a rare disease in childhood therefore it may represent a challenge to the clinician due to its resistance to anti-inflammatory treatment. Very often the initial aetiology often remains unclear. There are no specific laboratory parameters predicting the frequency or severity of the recurrences (6). Idiopathic recurrent pericarditis shares several features with auto inflammatory diseases. The findings in these patients suggest that idiopathic recurrent pericarditis may be an unrecognized auto inflammatory disease (3). Recurrences develop in up to 15% to 30% of adult patients with acute pericarditis. Recurrent pericarditis (RP) may be resistant to therapeutic interventions. The aetiology of the initial attack, and especially the cause of recurrences, often remain unclear. At the admission it might be complicated to exclude the presence of possible bacterial infection due to elevated *CRP* levels. The incidence of post-pericardiotomy syndrome is higher in children than in adults and may reach up to half of the cases of recurrent pericarditis in pediatric age group. Pneumonic infiltrations or pleural effusion found in up to 67% of patients with recurrent pericarditis and may reflect the same immune-mediated process that inflamed the adjacent pericardium, as no microbiologic aetiology could be verified (7). Respiratory tract infections are frequently found in patient records in cases of relapses. Persistent T-cell activation may be induced by antigens intrinsic to the epi-pericardium and cross react with viral antigens because of molecular mimicry (4). Genetic factors may contribute to recurrences of pericarditis (4) althow there was no positive family history for our patient. The non steroidal anti inflamatory drugs as ibuprofen, aspirin and indometacin are the commonly used first-choice treatment. The efficacy of corticosteroids in preventing relapses has been questioned, and warnings have been issued about steroid dependence and the possibility of more ferquent relapses in patients treated with corticosteroids (2). In our case relapses were were successfully treated with NSAIDs only. Colchicine is an effective drug in familial Mediterranean fever, but it has not been observed in the Latvian population so we have no our own experience with the use of colchicine in cases of RP. There are also some case reports from literature about a successful treatment of idiopathic recurrent pericarditis in children with interleukin-1beta receptor antagonist (anakinra) (7).The course of RP is frequently long and may be unpredictable, but overall prognosis seems to be good with only mild tamponade during the relapse and no data of further constrictive pericarditis(2,5) but recurrent pericarditis is an uncommon disease in children therefore there are rewiews of cases of this series in literature but not more detailed studies.

Conclusions. Recommended treatment of children with RP is primarily NSAIDs and the use of corticosteroids is only accepted in those with severe symptoms and only temporarily, if possible.



Picture 1. Massive pericardial effusions in echocardiography in performed in July, 2009 (A) and November 2009(B).



Picture 2. Marked cardiomegaly on the thoracic x-ray at the manifestation of the disease(A) and after the disaperance of the pericardial effusion (B).

Conflict of interest: None

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CASE REPORT

Torsion of the Diverticulum of the Appendix

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Summary
We report a case of a 1-year-old girl, operated on due to the symptoms of acute appendicitis. Torsion, necrosis and perforation of the large diverticulum of the *appendix vermiformis*, causing peritonitis were found during the operation. The complete luminary connection between appendix and the diverticulum was proved. Although appendix vermiformis itself was not the reason for the peritonitis, it was removed together with the diverticulum. The child's recovery after the operation was stable. The morphology revealed that the diverticulum of *appendix vermiformis* generally possessed features typical of a large intestine columnar epithelium, while its other layers, including a muscular layer and an irregularly expanded sub-mucosal layer, remained similar to the wall of the appendix. We suggest that the torsion of the true congenital diverticulum of the vermiform appendix mimicked acute appendicitis and caused peritonitis in the above-mentioned girl.
Key words: appendiceal diverticulosis in children, appendiceal diverticulitis, torsion of appendiceal diverticulum, congenital appendiceal diverticulum, peritonitis in children.

AIM OF THE DEMONSTRATION

Diverticula are rarely seen in the perinatal infant (5). Congenital appendicular diverticulum is an extremely rare phenomenon in the pediatric surgery (6,7). It has been observed more often in adult surgery, where the incidence of 0,014-2% of congenital v. acquired appendiceal diverticula has been recorded during appendectomies (6,8).

CASE REPORT

A 17-months-old girl presented to the emergency department of the children's hospital with a 3 day old high fever. She was seen by the general practitioner and received paracethamol. The girl was hospitalized with the diagnoses of a respiratory viral infection, pharyngitis and a urinary tract infection. Initial blood tests showed a remarkably elevated white blood cell (WBC) count (29,0x103/mm3), high levels of C-reactive protein (CRP) (183,0 mg/L) and a high erythrocyte sedimentation rate (90 mm/h). The urine test showed the presence of ketonemia (40 mg/dL). Parenteral rehydratation and antibacterial therapy with Cephotaxime were started. On the next day, the general condition of the girl had not improved. Hyperthermia and abdominal pain persisted. Abdominal echography showed a 27 to 46 mm round shaped mass in the ileocecal region but abdominal X-rays revealed the signs of ileus. At this point, the girl was referred to the surgeon and transferred for an emergency operation with the suspicion of an appendicular abscess.

The operation revealed peritonitis, localized in the ileocecal fossa and minor pelvis, and a round 31 to 52 mm mass, partially necrotic, fixed to the abdominal wall. Further inspection revealed that this mass was a diverticulum of the vermiform appendix, rotated by approximately 360°. Macroscopically, it was a typical digestive tract diverticulum with an intestine-like wall, *mesenteriolum* and a lumen connection to the appendix on the antimesenteric side (Fig.1). The appendix itself was 5 to 26 mm, not rotated but slightly bent at the diverticulum base and with a secondary inflammation (Fig.2). We performed a typical appendectomy and sent the specimen for the histopathology examination. We inspected 40-50 cm of the distal ileum and the ascending part of the colon, but the intestinal segments were undamaged. Recovery of the child after the operation was slow but stable, and required 12 days. The girl received parenteral antibacterial therapy with Cephtriaxone and Metronidazole postoperatively.

Histological sections were stained routinely with haematoxylin and eosin. The connection site between the diverticulum and appendix demonstrated features typical of the large intestine - a columnar epithelium with an abundance of crypts from the diverticulum side. The appendix showed an abundance of lymphatic noduli often with reactive centres, an upwardly orientated and partially damaged epithelium, a somewhat indistinct sub-mucosal layer and an absence of lamina muscularis mucosae. The lamina muscularis externa was reduced, although, the distinct subserosal layer was followed by a clearly detectable mesothelium. The structure of the diverticulum was not so homogenous, generally demonstrating features typical of the large intestine epithelium, but also a prominent thickening of the wall itself due to the increased sub-mucosal layer, with a subsequently reduced muscle layer and mesothelium. Small arteries with thickened walls and abnormally expanded lymphatic and blood capillaries were also characteristic for all the slides, but especially in those of the diverticulum wall.

DISCUSSION

The classification of appendicular diverticula divides them into the true or congenital, and false or acquired, depending on the presence or absence of the tunica muscularis in the diverticulum wall (1,3,4,6,7). Congenital appendicular diverticula further are divided into solitary or multiple (6,9). To our knowledge 7 cases of congenital appendicular diverticula (solitary and multiple) in children have been clearly reported in the literature so far; however, the real age of those children is not stated exactly (6).

The morphology revealed that the diverticulum of *appendix vermiformis* found during the operation has the wall structure characteristic of the congenital intestinal diverticulum. In general, it possesses features typical of the large intestine columnar epithelium while other of its layers remain similar to the wall of the appendix with an irregularly expanded sub mucosal layer. It may seem that this is an individual feature of the patient, possibly related to the development of the intestine in early embryogenesis. The appendix develops around the ninth embryonic week when a simultaneous event is the movement of herniated intestinal loops back into the abdominal cavity of the embryo. However, it may be more preferable to consider the endodermic epithelium and mesoderm relation disturbances under the influence of up-/down-regulated Hox genes which are known to regulate the development of the large intestine at that time (2). There is at least one report about the torsion of the appendiceal diverticulum producing the symptoms of acute appendicitis, as well as another report describing appendix diverticulum macroscopically which is surprisingly similarlike in its shape to the Meckel's-type diverticulum (6). Those two features were also observed in our case.

Usually, congenital appendiceal diverticula have been found during operations resulting from suspicions of acute appendicitis. There are some reports also about the radiologic diagnostic of appendiceal diverticulitis in pediatric and adult patients (4,7). We suggest, that not only an appendiceal diverticulitis, but also the torsion of the true congenital appendicular diverticulum should be considered as the differential diagnoses in the cases mimicking the symptoms of the acute appendicitis.

Conflict of interest: None

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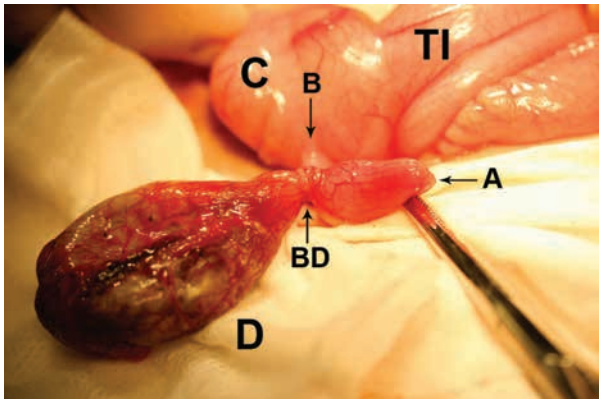

Fig. 1. Vermiform appendix with rotated and partially necrotic congenital diverticulum during appendectomy. (D) diverticulum, (BD) base of the diverticulum with the torsion, (A) apex of the appendix, (B) base of the appendix, (C) caecum and (TI) terminal ileum.

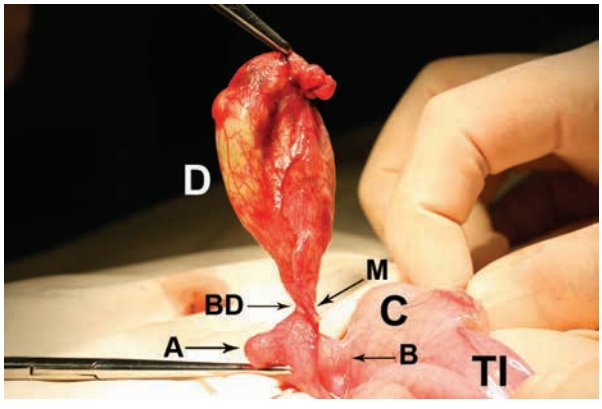

Fig. 2. View of the vermiform appendix and diverticulum during the operation after derotation. (D) diverticulum, (BD) base of the diverticulum on the antimesenteric side of the appendix, (M) mesenteriolum of the diverticulum, (A) apex of the appendix, (B) base of the appendix, (C) caecum and (TI) terminal ileum.

CASE REPORT

Spinal Cord Stimulation for Chronic Pain Relief: First Experience in Baltics

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Summary
We report the first case of spinal cord stimulator implantation in Baltics to patient with massive posttraumatic plexus lumbosacralis dxtr lesion, severe neuropathic pain syndrome and drug addiction problems. Follow-up time is 6 month since December 2011 and we have observed an obvious clinical and social improvement in patient status. Besides significant pain relief she has got employed and is tax payer instead of low-income person.
Key words: spinal cord stimulation, neuropathic pain, buprenorphine.

AIM OF THE DEMONSTRATION

The aim of demonstration is to demonstrate clinical efficacy of spinal cord stimulation and social benefits in a patient with long-term chronic neuropathic pain syndrome and drug abuse problems.

CASE REPORT

We studied 25 y.o. female patient after severe politrauma since car crash nine years ago. Diagnoses: Status post multiple pelvis fractures with plexus lumbosacralis damage (2001). Spinal abscess (laminectomy) surgery after epidural analgesia (2005). Osteomyelitis os ischii dxtr (2005). St post necrectomy due to IV grade bedsores. Free flap surgery on the buttocks. Unsuccessful trial to put in the spinal cord stimulation in Munich (Germany) (2008). Right hip arthroplasty with BHR prosthesis (2009).

Before the treatment she suffered from chronic neuropathic pain syndrome in the right leg, dislocation of right hip prosthesis and iatrogenic addiction to strong opioids (300 mg of oral morphine per day), haven`t been employed for several years and had status of low-income person.

She was admitted in the Hospital of Traumatology and Orthopaedics at the end of year 2010 and spinal cord stimulator implantation procedure was performed.

Octad (8-polar) electrode was implanted percutaneously on the right side of epidural space above dorsal horns of spinal cord with active electrodes on the right side at level Th 9. Recharchable pulse generator was implanted in the anterior abdominal wall. Variable programmes (A, B, C, D) for different activities were established. Pulse width varies from 180 to 249 milliseconds, pulse frequency from 40 to 60 megahertz, amplitude from 0.9 to 6.0 V. Pulse generator is sensitive to body position and change intensity of pulsation according to patient movements (Image 1).

Right hip joint revision arthroplasty was performed under general anaesthesia 2 weeks after spinal cord stimulator insertion. Postoperative pain relief was provided by wound catheter without opioids.

Body part covered by paraesthesia varies from the knee to the uncle in the one mostly used programme to all leg paraesthesia in another mostly used programme. Pain relief is limited to body parts covered by paraesthesia (Image 2). Median level of pain decrease from 6-8 to 0-3 by VAS score with breakthrough episodes 5 VAS. This pain was successfully treated by GABA agonists (pregabalin), NSAIDs, α_2-adrenergic agonist clonidin, tricyclic antidepressant amitriptilin. Patient was successfully involved in detoxication programme to reject use of strong opioids. Morphine was replaced by agonist-antagonist buprenorphine in decreasing doses (12-10-8-6-4 mg) per day, within 4 weeks. It has a high affinity for the μ opioid receptor, with slow dissociation, resulting in a long duration of action.

At the end of April, 2011 patient successfully underwent work interview, was examined by medical specialists, received approval for a not hard physical job and received permanent position in the supermarket company.

Patient is still under close supervision of surgeons, pain specialists and narcologist. Daily therapy includes gabapentin 600-900 mg per day, amitriptilin 50 mg per day and 8 mg of buprenorphine in divided doses.

DISCUSSION

Spinal cord stimulation differs from traditional surgery in several ways. The stimulator does not change the underlying condition; it simply changes the brain's perception of pain. Neurostimulators can treat chronic neuropathic pain using mild electrical impulses which are delivered to the spinal cord to mask the body's pain signals and replace them with a tingling

sensation. Studies have previously shown that spinal cord stimulation significantly reduces the pain score, concomitant medication use and sleeping problems in patients with chronic neuropathic pain syndrome, resistant to surgical and conservative treatment methods.

Neurostimulation for the treatment of chronic back and leg pain is clinically proven to provide effective and sustained pain relief (1-4), decrease use of opiate analgesics (1, 3, 4), and improve quality of life (2, 4) and functional ability (2, 4, 6).

Main indications for spinal cord stimulations are failed back surgery syndrome, complex regional pain syndrome, vascular disease and phantom limb pain (1, 2, 3, and 4). It`s possible to minimize or reject usage of strong opioids for pain relief. Patients significantly improve quality of life and are able to participate into the job market.

Neirostimulators with built-in sensor listens and learns from the individual patient's stimulation needs, then responds by automatically adapting settings each time the patient changes position. A recording memory mode remembers these changes for the future. The result for patients is a therapy which integrates more fully into daily life and movement.

The use of buprenorphine to treat chronic pain patients refractory to long - term opiate analgesic therapy is safe, effective, and well tolerated by these patients. The high-affinity blockade and the partial agonist ceiling confers a high safety profile clinically, a low level of physical dependence, and only mild withdrawal symptoms on cessation after prolonged administration (7).

Conflict of interest: None

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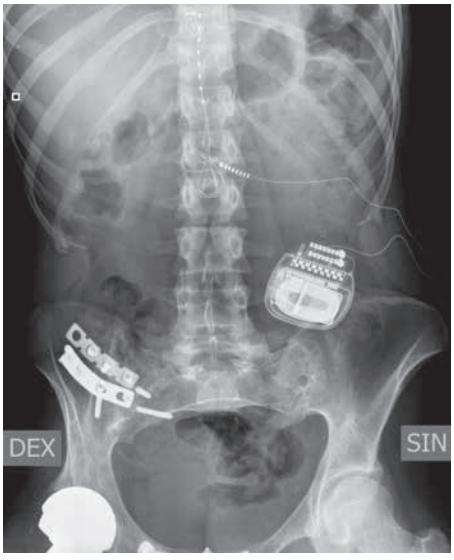

Image I. X-ray AP view

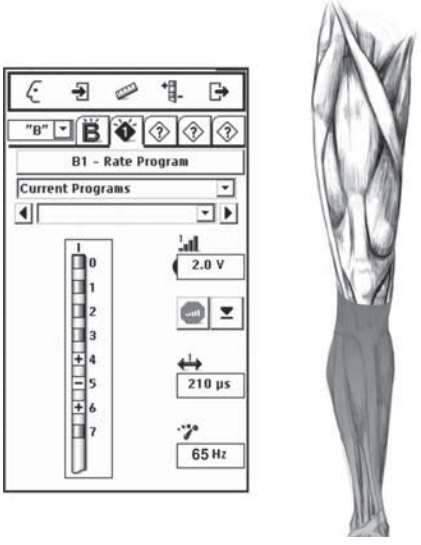

Image II. Programmer screen image; patient`s area, covered by paraesthesia

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