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Rīgas Stradiņa universitāte

Liāna Švampāne

CHARACTERIZATION OF HEREDITARY ENDOMETRIAL CANCER IN LATVIA

Summary of Doctoral Thesis
for obtaining the degree of a Doctor of Medicine

Speciality – Oncology

Riga, 2015

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Contents

LIST OF ABBREVIATIONS

CI – confidence interval

DNS – deoxyribonucleic acid

FIGO – International Federation of Gynecology and Obstetrics

G – *Grade*; degree of tumour differentiation

HEC – hereditary endometrial cancer

HEC susp – suspected hereditary endometrial cancer

HNPCC – hereditary nonpolyposis colorectal cancer

IAfRoC – International Agency for Research on Cancer

MMR – mismatch repair

MSI – microsatellite instability

PCR – polymerase chain reaction

SEC – sporadic endometrial cancer

INTRODUCTION

According to the Centre of Health Economics of Latvia data on the proportion of malignant tumours in Latvia in 2009, endometrial cancer is found in 6.9% cases of women or 29.02 per 100,000 women, whereas 4.7% or 10.72 per 100,000 women have died. The percentage of cases per stage of disease in Latvia is, on average, 57.8% in stage I, 12.6% in stage II, 13.6% in stage III, 7.0% in stage IV, 9.0% – without a stage (Centre of Health Economics of Latvia, 2010).

Endometrial cancer is the fourth most common cancer localization for women in many developed countries. For example, according to Cancer Research UK, 8,475 new cases have been diagnosed in 2011 in the UK which was the fourth place among all the cancer localizations for women. Endometrial cancer incidence in the UK is relatively low in the European Union, but it is the highest in Slovakia, Czech Republic and Latvia (Cancer Research UK, 2014).

According to the International Agency for Research on Cancer, year 2008 saw the highest endometrial cancer incidence in the world in recent years, i.e. 288,387 new endometrial cancer cases or 8.2 per 100,000 people. There were 73,854 endometrial cancer deaths in the same year or 2.0 per 100,000 people. In 2008 the incidence in more developed parts of Europe was 93,562 new cases (12.3 per 100,000 people), while in less developed parts of Europe it was 144,869 cases (5.9 per 100,000 people) (IAfRoC, 2010). In Latvia there were 383 new endometrial cancer cases (18.6 per 100 000 people), while 82 women had died from endometrial cancer (2.8 per 100,000 people) (Centre for Disease Prevention and Control of Latvia, 2012).

Endometrial cancer incidence increases for women in the menopause and postmenopause age up to 75%. The mean age at disease onset is from 55 to

65 years (DiCristofano and Ellenson, 2007). For women under 40 years old, endometrial cancer is diagnosed in 2–5% of all endometrial cancer cases. In the last two years a trend of decreasing average age of onset has been observed to about 50–60 years.

Five-year survival rate in Latvia for all stages together is 58.8%, being 86% in stage I but just 12% in stage IV (Cancer Patient Registry of Latvia, 2008). The effectiveness of therapy and survival is affected by the morphological form of the tumour, low degree of differentiation or high grade, negative hormone receptors, aneuploid tumours, or positive peritoneal cytology.

Evaluating all endometrial cancer disease indicators and risk factors creates a necessity for earlier endometrial cancer detection, radical therapy or a way to prevent the development of cancer. Therefore, appropriate preventive measures by establishing an endometrial cancer risk group are one of the most promising directions in oncology.

In a longer time period, many scientists and researchers are studying oncological diseases with respect to heredity where several members of the same family have been diagnosed with different cancer localizations over multiple generations, including hereditary endometrial cancer (HEC), 2–5% of all diagnosed endometrial cancers (Watson et al., 1993).

One of the most studied cancers is hereditary nonpolyposis colorectal cancer (HNPCC). The connection of several genes to the development of HNPCC has been proved, as well as other cancer localizations related to this syndrome. In these families the second most common cancer localization after colorectal cancer is endometrial cancer, increasing the lifetime risk of getting endometrial cancer by 40–60% in relation to this syndrome (Watson et al., 1993, Vasen et al., 1994; Aarnio et al., 1999, Parc et al., 2000; Prat et al., 2007). Research carried out over the last years indicates that another HEC

group exists in which female first- and second-degree relatives over several generations have certain gene mutations associated with HEC (Sandles et al., 1992., Gruber and Thompson, 1996; Lurie et al., 2011).

Until now all studies on hereditary endometrial cancer were performed in different geographical areas covering multiple ethnic groups which resulted in differences in clinical and molecular characteristics. Therefore, it is very important to perform such a study in Latvia to analyse the clinical and molecular characteristics of hereditary endometrial cancer. Study results are very important in developing diagnosis and therapy recommendations most appropriate for Latvian patients and people in the risk group. Implementing these recommendations would be very important in treating oncological diseases and improving cancer prevention in Latvia.

Aim

To determine the clinical and molecular characteristics of endometrial cancer patients in Latvia.

Enabling objectives

1. To study the clinical characteristics of sporadic and hereditary endometrial cancer by analysing oncological family history data for endometrial cancer patients in Latvia.
2. To determine the incidence of hereditary endometrial cancer in Latvia.
3. To determine predisposing mutations in families with endometrial cancer cases.
4. To determine *MSH2* and *MSH6* gene expression in sporadic and hereditary endometrial cancer cells with immunohistochemical methods.
5. To determine survival in sporadic and hereditary cancer patient groups.

Novelty

The clinical and molecular characteristics of hereditary endometrial cancer were determined in Latvia for the first time**.** The effectiveness of the classic diagnostic criteria of the hereditary nonpolyposis colorectal cancer syndrome (Amsterdam criteria) as well as the adapted diagnostic criteria of hereditary endometrial cancer syndrome in identifying both hereditary nonpolyposis colorectal cancer syndrome and hereditary endometrial cancer syndrome was proven to be very limited. The diagnostic criteria of late-onset hereditary endometrial cancer syndrome were approved which also effectively reveal the diagnostics of hereditary endometrial cancer.

Practical application

Study results confirmed that all endometrial cancer patients should gather their oncological family history. By analysing oncological family history, families with increased risk should be identified that correspond to the late-onset hereditary endometrial cancer criteria. Cancer-afflicted family members in this group should be offered full *MLH1, MSH2* and *MSH6* gene examination. In families with proven *MLH1*, *MSH2* and *MSH6* gene mutations, all healthy relatives of the patients are recommended doing molecular investigations. All afflicted and healthy mutation carriers should undergo a particular preventive measures programme. In families with no proven HEC and HNPCC syndrome related mutations all female family members should undergo a preventive measures programme. Using this knowledge in everyday clinical practice would considerably improve early endometrial cancer diagnosis and therapy results in Latvia.

Theses

1. The significance of classical Amsterdam criteria in hereditary endometrial cancer diagnosis is limited.
2. There is a potentially characteristic HEC syndrome mutation variation in Latvia – *MLH1*, *MSH2*, and *MSH6*.
3. Families with HNPCC and HEC syndrome have phenotypical expressions of cancer development characteristic to HEC.
4. The lack of protein expression in the *MSH6* gene during immunohistochemical examination can be used as a criterion for determining the carriers of the mutations constituted by the *MSH6* gene in a group of consecutive hospitalized endometrial cancer patients in Latvia.

1. MATERIALS AND METHODS

The methodology of this study has been approved by the ethics committee of Rīga Stradiņš University.

1.1. General description of patients

704 consecutively hospitalized patients diagnosed with endometrial cancer were included in a prospective study from January, 2006 to April, 2009 at Riga Eastern Clinical University Hospital, Latvian Oncology Centre. All 704 patients were included in a study of the clinical and molecular characteristics of hereditary endometrial cancer during which their oncological family histories were obtained. Cases were considered consecutive if at least 70% of new endometrial cancer patients were included in the study from the specific hospital in a particular time period. The main inclusion criteria were patients' written consent to take part in the study, provide a blood sample for molecular examination and postoperative material for immunohistochemical examination to examine the tissues genetically. Detailed information on the course of disease, its morphological characteristics and other necessary indicators were obtained from medical histories, ambulatory medical records and the Centre of Health Economics of Latvia.

All patients had their diagnosis verified histologically by performing fractional abrasion and histologically examining the postoperative material.

Patients who participate in the study cover all age groups with no age restriction. The age range was from 30 to 85 years.

1.2. Research methods

1.2.1. Analysis of family medical history

Standardized oncological family histories were collected from all the patients (see Table 1.1.). They had to answer the following questions:

1. Have any of your relatives (mother, father, grandparents, brothers, sisters, children, grandchildren, uncles, aunts) had malignant tumours?
2. What was the tumour localization from the specified group of relatives?
3. At what age was the tumour detected?

If necessary, additional questions were asked about the affected family members on the applied methods of treatment (e.g., surgery, radio- or chemotherapy) and other questions to specify the tumour diagnosis and localization.

Oncological family histories were analysed using both internationally approved as well as modified hereditary cancer diagnostic criteria (see Table 1.1). Patients whose oncological family histories corresponded with the diagnostic criteria of hereditary cancer were labelled as belonging to the hereditary cancer group for the purpose of this study. Patients who did not have any malignant tumour cases in their oncological family history were labelled as belonging to the sporadic cancer group. Likewise, if one or multiple different localization cancer cases had been identified in an oncological family history, but the family tree did not correspond with any of the diagnostic criteria used in the study, then the case was marked as sporadic.

Table 1.1

Hereditary endometrial cancer diagnostic criteria

No.	Inherited syndrome	Diagnostic criteria
1.	Site-specific HEC	At least three first-degree relatives with endometrial cancer; at least one of the cancers diagnosed before age 50.
2.	Late-onset HEC	Three first -degree relatives with endometrial cancer at any age
3.	HNPCC	Amsterdam I criteria: 1) at least three relatives with colorectal cancer, at least one should be a first-degree relative to the other two; 2) colorectal cancer in at least two consecutive generations; 3) at least one cancer case diagnosed before age 50; 4) familial adenomatous polyposis must be ruled out; 5) tumours must be histologically confirmed
4.	Late-onset HNPCC	Amsterdam II criteria with no age restriction: 1) at least three relatives with an HNPCC associated cancer (colorectal, endometrial, small bowel, renal pelvis, ureteral), at least one should be a first-degree relative of the other two; 2) cancer cases in at least two consecutive generations; 3) at least one cancer case diagnosed before age 50; 4) familial adenomatous polyposis must be ruled out; 5) tumours must be histologically confirmed.
5.	Sporadic cancer	1) One or two different localization cancer cases for first- and second-degree relatives; 2) No previous cancer cases in the family

If eligibility to the diagnostic criteria of hereditary endometrial cancer syndrome was recognized by examining oncological family history, a patient's first-degree relatives were invited to the research unit of Rīga Stradiņš University, Hereditary Cancer Institute where she could receive doctor's advice, written information on the clinical characteristics of the hereditary cancer syndrome found in her family and prophylactic recommendations.

After informing a patient about the nature of the study and obtained her written consent, 6 ml of peripheral venous blood were collected from the patient. The blood sample was stored at a +2–8 °C temperature and its DNA was isolated in a seven day period.

Blood samples were collected from all 704 (100%) patients.

Paraffin embedded tissues from the endometrium were collected from 109/704 (15.5%) for immunohistochemical examination.

1.2.2. Molecular examinations

MLH1, *MSH2* un *MSH6* gene DNA examination was done for patients whose oncological family histories corresponded with the HEC and HNPCC diagnostic criteria. Overall molecular examinations were performed for 11/19 (57.9%) patients from the hereditary group who corresponded with the hereditary cancer diagnostic criteria.

1.2.3. DNA isolation

Qiagen FlexiGene DNA Kit was used to isolate DNA from the blood according to the manufacturer's instructions. DNA quality was checked using 1% agarose gel with the following visualization and digital documentation. UV-Vis spectrophotometer Nanodrop 1000 was used to measure DNA concentration.

1.2.4. Polymerase chain reaction

Polymerase chain reaction (PCR) was used to obtain DNA fragments for sequencing. Previously described primers were used in the PCR (Kolodner et al., 1994, 1995, 1999), optimizing conditions.

1.2.5. PCR with a fluorescent dye

Primers described by Kolodner et al. (1994, 1995, 1999) were used for a PCR reaction with Applied Biosystems Big dye v3.1. fluorescent dye. Qiagen MinElute 96UF PCR Purification Kit was used to purify the PCR (50µl) fragment.

1.2.6. Capillary electrophoresis

Capillary electrophoresis was done by the Applied Biosystems genetic analyser AB13130 as per Applied Biosystems instructions, using a 36 cm capillary and POP-7 polymer under standard conditions.

1.2.7. Sequence analysis

Sequence analysis was done by Applied Biosystems SeqScape and Seqencing Analysis software which identify nucleotides, allow to assess the quality of data and an initial visual assessment of mutations and the existence of frameshift mutations as well as compare sequences against reference sequences. SeqScape automatically determines possible SNP mutations and identifies places where frameshift mutations have occurred. Several databases were used for data interpretation – NCBI SNP, HGMD, Insight-group.

Immunohistochemical examination of endometrial normal and cancer tissue was done in 109/704 (15.5%) cases to determine *MSH2* and *MSH6* gene expression. The immunohistochemical group contained cases for which the healthy and cancer tissue samples obtained during surgery were available at the pathology unit of the hospital.

636/704 (90.4%) patients received surgical therapy during which healthy and cancer tissues were collected. The immunohistochemical examination group included cases from the study after radical surgeries.

1.2.8. Immunohistochemical method

The molecular characteristics of the patient groups were examined immunohistochemically. The avidin-biotin-peroxidase method (Hsu et al., 1981; Kiernan, 1999) was used to examine endometrial tissue samples. The tissue samples were initially embedded into paraffin and cut into 4μm portions. They were then incubated at room temperature at a 1:1000 dilution of rabbit

antiserum for 60 minutes. Afterwards the samples were rinsed in three changes of phosphate buffer solution and incubated at room temperature in biotinylated secondary antiserum, goat to rabbit IgG, diluted 1:40. Next the avidin-biotin complex solution was prepared by mixing avidin and biotinylated horseradish peroxidase solutions. After the cut portions were rinsed in three changes of phosphate buffer solution, they were incubated at room temperature in a avidin-biotin complex solution for 60 minutes. Then they were rinsed in three changes of phosphate buffer solution. A visualization reaction was performed, the samples were rinsed with distilled water and counterstained for three minutes. Cover glass was placed over the samples and they were examined under a microscope. The results were grouped as follows: 0 – negative (lack of protein – pathology); 1 – weak expression; 2 – medium expression (no mutation); 3 – intensive expression (no mutation); F – focal expression; F1 – focal or weak expression (mutation suspected).

1.2.9. Statistical analysis methods

Confidence intervals. 95% Wilson confidence intervals (Wilson, 1927) were constructed to analyse the data. The confidence intervals were computed for patient groups, i.e. for the sporadic and hereditary cancer groups and for each hereditary cancer subgroup. Wilson's method was chosen because these intervals are not so much affected by the total amount of patients in each group, the proportion of some patients against the total amount of patients in the group, as well as the intervals covering only positive values (Agresti and Coull, 1998). As there were not many patients in the HEC group, these considerations were important.

Survival analysis. Survival in the sporadic and hereditary cancer group was analysed using GraphPad Prism 5 software and the Kaplan-Meier method. The p-value was computed using the Mantel-Cox test. A p-value of less than 0.05 was considered statistically significant pointing to a higher probability that

differences in survival between the sporadic and hereditary cancer group are not by chance. The hazard ratio for the sporadic and hereditary group was also computed showing which of the two groups had a higher mortality risk during the study period.

2-sample test for equality of proportions without continuity correction was carried out and p-values were computed in R software to determine the differences between the sporadic and hereditary cancer groups in terms of cancer activity, age at disease onset, stage at diagnosis and tumour degree of differentiation. Mann-Whitney U test was performed to compare the differences in age at death between the sporadic and hereditary cancer groups.

2. RESULTS

2.1. Clinical characteristics

By analysing oncological family histories for the 704 patients with histologically confirmed endometrial cancer, the obtained results were grouped according to diagnostic criteria. 19/704 (2.7%) cases were hereditary cancer. In 7/704 (0.99%) cases oncological family history corresponded with site-specific HEC criteria. Late-onset hereditary endometrial cancer was found in 3/704 (0.43%) cases. Also separately grouped were the patients whose oncological family history corresponded with Amsterdam criteria I (HNPCC) and Amsterdam criteria II (late-onset HNPCC). Thereby, the HNPCC syndrome had 4/704 (0.57%) cases while 5/704 (0.71%) cases were late-onset HNPCC. In the sporadic cancer group 685/704 (97.3%) patients had first diagnosed endometrial cancer and did not correspond with HEC or HNPCC (see Table 2.1).

Table 2.1

Clinical incidence of the HEC syndrome

Hereditary syndrome	Amount	%
Site-specific HEC	7	0.99
Late-onset HEC	3	0.43
HNPCC	4	0.57
Late-onset HNPCC	5	0.71
Sporadic cancers	685	97.3
Total	704	100

In the sporadic group we also included patients whose families did not correspond with the classical Amsterdam criteria but had another endometrial cancer case in the family for a first- or second-degree relative at any age. These cases may be labelled as suspected hereditary endometrial cancers. There were

78/685 (11.4%) such families in the sporadic cancer group, while in 607/685 (88.6%) families another endometrial cancer case was not present.

Patients in this study covered all age groups from 30 to 85 years. Seven patients whose diagnostic criteria corresponded with site-specific HEC were between 48 and 72 years old. The mean age of onset was 60.6 years.

In the hereditary group one of the four patients in the HNPCC subgroup was 35 years old at disease onset but the age range was from 35 to 70 years.

Overall, the hereditary group included 15.8% of women under age 50. The sporadic group included 6/685 (0.9%; 95% CI: 0.4–1.9%) cases up to age 40 and 52/685 (7.6%; 95% CI: 5.8–9.8%) cases up to age 50. 84.2% of patients in the hereditary group were up to age 50 where 3/19 (15.8%; 95% CI: 5.5–37.6%) cases were up to age 60, 8/19 (42.1%; 95% CI: 23.1–63.7%) cases were up to age 60, while 5/19 (26.3%; 95% CI: 11.8–48.8%) cases were up to age 70 (see Table 2.2).

Table 2.2

Age at disease onset and its 95% confidence interval for the hereditary and sporadic groups' patients by diagnosed syndrome

Diagnosed syndrome/age (years)	**31–40 Amount (%)**	**41–50 Amount (%)**	**51–60 Amount (%)**	**61–70 Amount (%)**	**71+ Amount (%)**
Hereditary cancers	1 (5.3)	2 (10.5)	3 (15.8)	8 (42.1)	5 (26.3)
Sporadic cancers	6 (0.9)	52 (7.6)	193 (28.2)	236 (34.4)	198 (28.9)
95% CI: hereditary cancers	0.9–24.6	2.9–31.4	5.5–37.6	23.1–63.7	11.8–48.8
95% CI: sporadic cancers	0.4–1.9	5.8–9.8	24.9–31.7	31.0–38.1	20.2–25.8

The mean age at disease onset for patients in the suspected HEC subgroup was 60 years while their first- or second-degree relatives' mean age at disease onset was 57 years. Compared with the hereditary cancer group, there is not a statistically significant age difference and most patients are aged between 51 and 70 years (see Figure 2.1.).

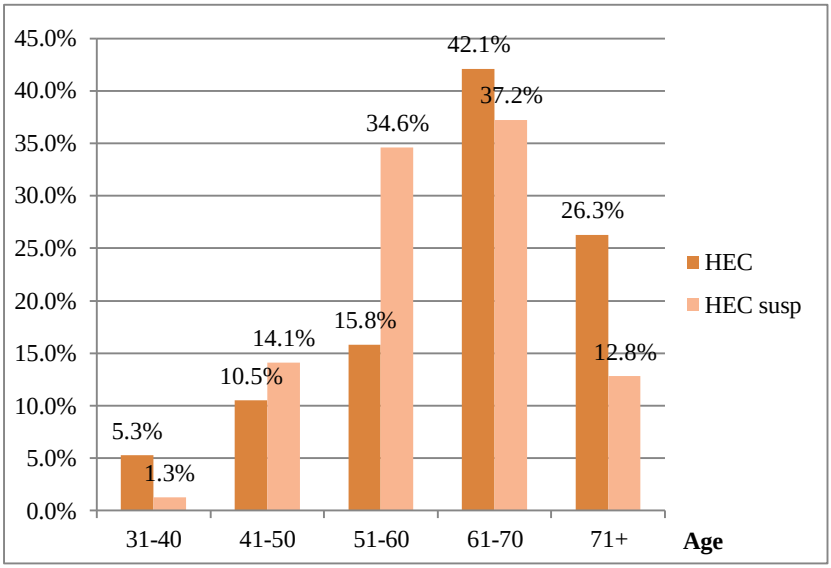

Figure 2.1. Mean age at disease onset at the hereditary and suspected hereditary cancer groups

The study included all four stages of cancer which were diagnosed postoperatively in 636/704 (90.4%) cases. In 68/704 (9.6%) the stage of disease could not be determined because the patients could not be operated on due to comorbidities (see Table 2.3).

Table 2.3

Classification of patients included in the study by the stage of disease with 95% CI

Stage	I		II		III		IV		Unstaged	
Amount/ %	437	62.1	88	12.5	95	13.5	16	2.3	68	9.6
95% CI	58.4–63.6		10.3–15.1		11.2–16.2		1.4–3.7		7.7–12.1	

15/19 (78.9%; 95% CI: 56.7–91.5%) patients were diagnosed in stage I and 1/19 (5.3%; 95% CI: 0.9–24.6%) in stage II. In 3/19 (15.8%; 95% CI: 5.5–37.6%) cases the stage could not be determined as no surgery was performed. According to the diagnostic criteria, there were no cases diagnosed in stage III and IV. In the sporadic cancer group, taking the total number of patients (704 patients) into account, four stages of disease were represented (see Table 2.4).

Table 2.4

Classification of the hereditary and sporadic group by the stage of disease at disease onset with 95% CI

Stage of disease	Hereditary group (%)	95% CI	Sporadic group (%)	95% CI
I	15 (78.9)	56.7–91.5	419 (61.2)	57.5–64.7
II	1 (5.3)	0.9–24.6	97 (14.2)	11.7–17.0
III	-	-	92 (13.4)	11.1–16.2
IV	-	-	17 (2.5)	1.6–3.9
Unstaged	3 (15.8)	5.5–37.6	60 (8.7)	6.9–11.1
Total	19 (100)		685 (100)	

The greatest number of patients in the suspected HEC subgroup was in stage I – 52/78 (66.7%) of patients, because most patients that were examined were in stage I. Differences arose in stages III and IV as there were no cases in these stages for the hereditary group, whereas the suspected HEC group had 8 (10.3%) patients in stage III and 3 (3.8%) patients in stage IV. With respect to the sporadic group, the data are similar to the hereditary case (see Figure 2.2).

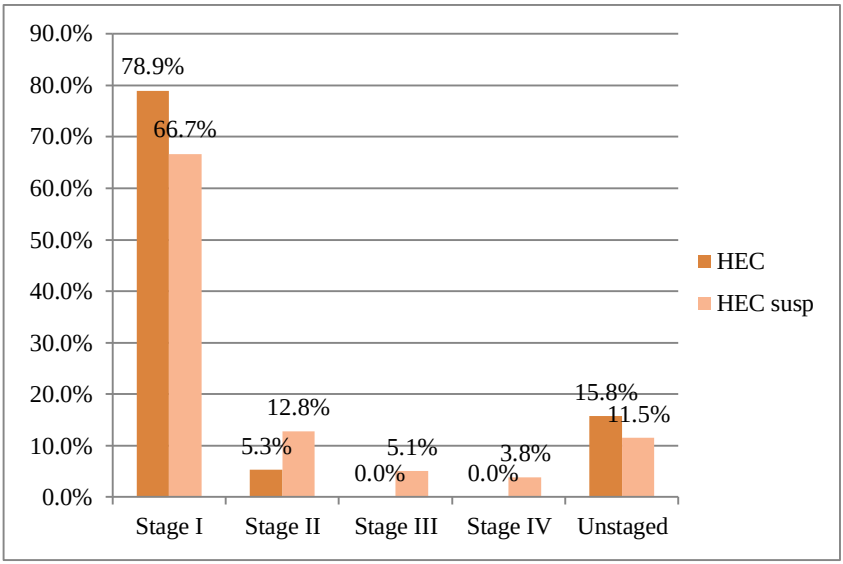

Figure 2.2. Stages of cancer in the hereditary and suspected hereditary cancer groups

Looking at the degree of differentiation, more patients' cancers were highly differentiated both in the hereditary and sporadic groups (p = 0.70).

Comparing the degree of differentiation of the hereditary and sporadic groups, no significant differences were observed (see Table 2.5).

Table 2.5

Classification of the hereditary and sporadic groups by the degree of tumour differentiation with 95% CI

Degree of differentiation	Hereditary group (%)	95% CI	Sporadic group (%)	95% CI
G1	6 (31.6)	1.4–54.0	189 (27.6)	24.4–31.1
G2	11 (57.9)	36.3–76.9	372 (54.3)	50.6–58.0
G3	2 (10.5)	5.7–51.0	98 (14.3)	11.9–17.1
Undetermined	-	-	26 (3.8)	2.6–5.5
Total	19 (100)		685 (100)	

According to the degree of differentiation, the data are similar in the case of hereditary and suspected HEC groups compared with the sporadic cancer group (see Figure 2.3).

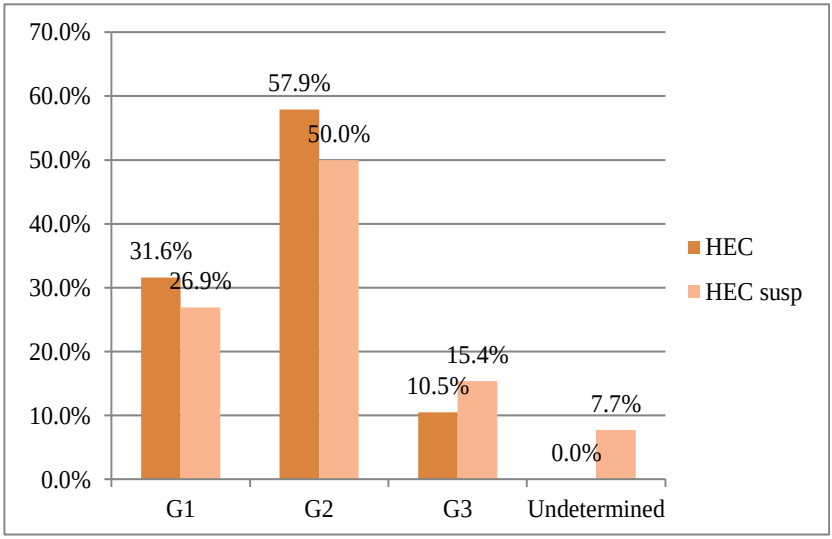

Figure 2.3. Degrees of differentiation in the hereditary and suspected hereditary cancer groups

To assess the activity of cancer in a 4–5 year period, the following data were obtained from patients whose endometrial cancer was diagnosed in 2006 and 2007. 206/704 (29.3%; 95% CI: 84.1–92.2%) patients from the sporadic group participated in the study in 2006. 5/206 (2.4%; 95% CI: 0.9–4.9%) patients had recurrent endometrial cancer, whereas 18/206 (8.7%; 95% CI: 5.0–11.9%) patients had various metastases (lymphogenic, bone, pulmonary). In 3/206 (1.5%; 95% CI: 0.2–3.1%) patients a second cancer localization was found which was unrelated to endometrial cancer.

Similar results were obtained in 2007 when the study had 210/704 (29.8%; 95% CI: 84.8–92.7%) cases of first diagnosed endometrial cancer. 8/210 (3.8%; 95% CI: 1.5–6.6%) patients had recurrent cancer, while 13/210 (6.2%; 95% CI: 3.3–9.2%) patients had lymphogenic, bone and liver metastases. A second cancer localization was found in 4/210 (1.9%; 95% CI: 0.7–4.4%) patients (see Table 2.6).

Table 2.6

Cancer progression and other cancer localizations in the sporadic cancer group for patients included in 2006 and 2007 three years after primary therapy, 95% CI

Sporadic cancer dg year	No. of patients	%	Re-currences	%	Me-tas-ta-ses	%	2nd local-iza-tion	%
2006	206	29.3	5	2.4	18	8.7	3	1.5
95% CI	84.1–92.2		0.9–4.9		5.0–11.9		0.2–3.1	
2007	210	29.8	8	3.8	13	6.2	4	1.9
95% CI	94.8–92.7		1.5–6.6		3.3–9.2		0.7–4.3	

Comparing the hereditary group with the sporadic group, it can be seen that recurrences were more frequent in the hereditary group – 5.3% (95% CI: 0.9–24.6%) vs 3.1% (95% CI: 1.8–5.3%) cases ($p = 0.61$). Metastases were approximately three times more frequent in the hereditary group than in the sporadic group as well – 26.3% (95% Cl: 11.8–48.8%) vs 7.5% (95% CI: 5.3–10.4%) cases ($p < 0.01$). Another cancer localization was found in 3/19 (15.8%; 95% CI: 5.5–37.6%) cases in the hereditary group and 7/416 (1.7%; 95% CI: 0.8–3.4%) cases in the sporadic group ($p < 0.01$). Disease progress or metastases were not observed in 10/19 (52.6%; 95% CI: 31.7–72.7%) patients in the hereditary group and 365/416 (87.7%; 95% CI: 84.2–90.6%) patients in the sporadic group ($p < 0.01$; see Table 2.7).

Table 2.7

Cancer episodes in the hereditary and sporadic cancer groups with 95% CI

Cancer episodes	Hereditary cancer (%)	95% CI	Sporadic cancer (%)	95% CI
Recurrences	1 (5.3)	0.9–24.6	13 (3.1)	1.8–5.3
Metastases	5 (26.3)	11.8–48.8	31 (7.5)	5.3–10.4
Another cancer localization	3 (15.9)	5.5–37.6	7 (1.7)	0.8–3.4
No new cancer episodes	10 (52.6)	31.7–72.7	365 (87.7)	84.2–90.6
Total	19 (100)		416 (100)	

From January, 2006 to April, 2010 (which is the duration of the study) 122/704 (17.3%) patients died (see Table 2.8).

Table 2.8

Deceased patients in all endometrial cancer stages in HEC and SEC groups together with 95% CI

Stage	I	II	III	IV	No stage
Amount of patients (%)	38 (5.4)	19 (2.7)	25 (3.5)	11 (1.6)	29 (4.1)
95% CI	4.0–7.3	1.7–4.2	2.4–5.2	0.9–2.8	2.9–5.9

102/122 (83.6%) patients died from recurrent endometrial cancer or its metastases, while 3/122 (2.5%) patients died from another localization cancer which was diagnosed during the control period. 17/122 (13.9%) patients died from comorbidities unrelated to oncology. Of the 19/704 (2.7%) patients from the hereditary group included in the study, 6/122 (4.9%) have died. One patient was from the site-specific HEC subgroup but two were from the late-onset HEC subgroup. Another patient was from the HNPCC subgroup but two patients – from the late-onset HNPCC subgroup. Two patients died from the spreading of another localization cancer. Four patients died from recurrent endometrial cancer and the metastases caused by it.

6/78 (7.7%) patients who died were from the suspected endometrial cancer subgroup which belonged to the sporadic cancer group. All six patients died from complications caused by endometrial cancer and disease progress.

Almost a half of all the deceased patients had a survival of up to 24 months, i.e. 59/122 (48.4%), including one patient from the HEC group 37/122 (30.3%) survived up to 12 months, including three patients from the HEC group (HNPCC – 1, late-onset HNPCC – 2). 17/122 (14.0%) patients had a survival of up to 36 months, including two patients from the HEC group (HEC – 1, late-onset HEC – 1). A survival of more than 36 months was registered for just 3/122 (2.4%) patients (see Figure 2.4).

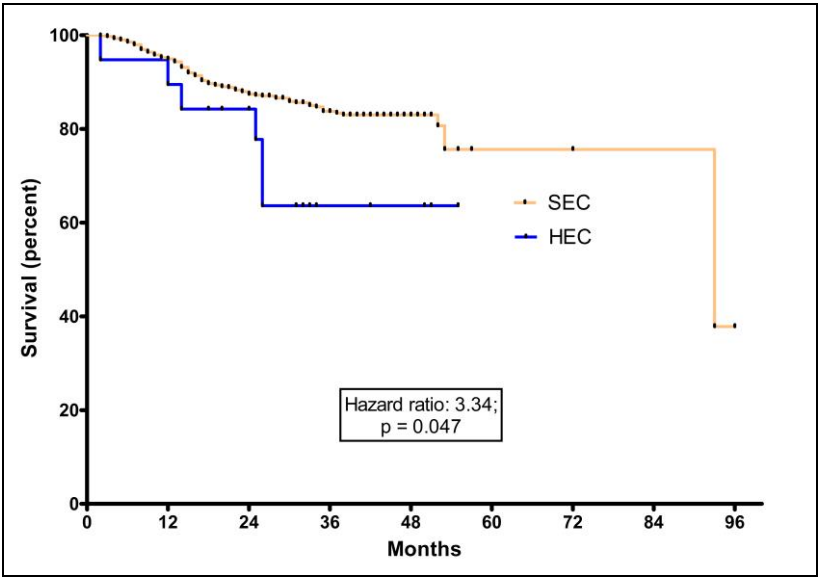

Figure 2.4. Survival for the hereditary and sporadic cancer groups

During the final period of the study data were collected from the Centre for Disease Prevention and Control of Latvia regarding all the patients included in the study that died up to 1 December 2013. 204/704 (29.0%) patients have died from January 2006 to December 2013 where 195/685 (28.5%) were from the sporadic group and 9/19 (47.4%) were from the hereditary group. The breakdown is as follows – 2/19 (10.5%) HEC patients, 2/19 (10.5%) late-onset HEC patients, 2/19 (10.5%) HNPCC patients and 3/19 (15.5%) late-onset HNPCC patients. For all patients in the hereditary group death was associated with recurrent cancer or metastases.

2.2. Molecular characteristics

Overall 11/19 (57.9%) patients of the hereditary group gave consent to molecular examination of their blood samples of which 7/19 (36.8%) patients corresponded with site-specific HEC and 4/19 (21.1%) patients corresponded with HNPCC according to the diagnostic criteria. Gene mutations were found in 6/11 (54.5%) site-specific HEC cases and 2/11 (18.1%) HNPCC cases. In 3/11 (27.4%) cases no gene mutations were found. Molecular examination was not performed for suspected HEC patients.

One HNPCC patient had the missense mutation P640S (rs63749792) in the *MLH1* gene. Another patient from the HNPCC subgroup had the splice site mutation IV5+A>T in the *MSH2* gene.

Four HEC patients had the following mutations: two carriers of frameshift mutations in the *MSH6* gene 2150TCAG (rs63750159) and 1050delC, two carriers of missense mutations in the *MLH1* (I219V (rs1799977)) and *MSH2* (G322D (rs4987188)) genes.

Two patients had no clinically putative significant mutations.

The frequency of site-specific HEC and HNPCC gene mutations in hospitalized endometrial cancer patients in Latvia is 0.9%.

Molecular characteristics of the immunohistochemically examined group are described further. By doing a repeated examination of endometrial tissues, no cancer tissues were found in 2/113 (1.8%) cases. In other 2/113 (1.8%) cases, serious technical difficulties were encountered during immunohistochemical examination, which prevented assessing the examination results. In 109/113 (96.4%) cases the expression of *MSH2* and *MSH6* proteins was analysed in both the healthy and the cancer tissues.

Immunohistochemical examination for the available tissue samples was performed for 7/19 (36.8%) hereditary group patients and 95/685 (13.9%) sporadic group patients. Negative protein expression of the *MSH2* gene was found in 14/102 (13.7%) cases in the sporadic cancer group. Negative protein expression of the *MSH2* gene was not found in the 3/7 (42.8%) examined site-specific HEC patients, whereas negative protein expression of the *MSH2* gene was found in 1/7 (14.3%) late-onset HEC patient of the 3/7 (42.8%) examined late-onset HEC patients. In 23/102 (22.5%) cases negative protein expression of the *MSH6* gene was found in the sporadic group.

Looking at the available tissue samples in the hereditary group, three site-specific HEC patients had no negative protein expression of the *MSH6* gene, while negative protein expression of the *MSH6* gene was found in 1/7 (14.3%) late-onset HEC patient of the three examined late-onset HEC patients. No tissue samples with the HNPCC syndrome were available. 1/7 (14.3%) cases with late-onset HNPCC was examined in which negative protein expression was found in the *MSH6* gene but not *MSH2* gene. 8/102 (7.8%) cases of the sporadic group had both negative protein expression of the *MSH2* and *MSH6* genes (see Table 2.9.).

Table 2.9.

Breakdown of negative protein expression cases of the *MSH2* and*MSH6* genes in the hereditary and sporadic cancer groups

Diagnostic criteria	Tissue samples available	Technical difficulties	Examined cases	*MSH2* protein expression		*MSH6* protein expression	
				Norm.	Negat.	Norm.	Negat.
Site-specific HEC	3	-	3	3	-	3	-
Late-onset HEC	3	-	3	2	1	2	1
HNPCC	-	-	-	-	-	-	-
Late-onset HNPCC	1	-	1	1	-	-	1
Sporadic cancer	104	2	102	88	14	79	23
Total	109	2	109	94	15	84	25

In the sporadic group, excluding the suspected HEC subgroup, immunohistochemical analysis was performed in 21/78 (26.9%) cases to determine the expression of the *MSH2* and *MSH6* gene proteins. Negative *MSH2* gene protein expression was detected in 1/78 (1.3%) cases, negative *MSH6* gene protein expression was found in 3/78 (3.8%) cases, while negative protein expression of both *MSH2* and *MSH6* genes was found in 3/78 (3.8%) cases. No gene protein expression was found in 14/78 (66.7%) cases.

2.3. Family trees

Patient family tree analysis was done to assess the data of the patients included in the study. Family tree creation has historical significance but they are still important nowadays. Family tree analysis is especially important in the case of hereditary diseases. Special software exist which are used for drawing family trees and analysing data.

2.3.1. Family trees of a HEC-positive family

1st case – patient R (ID No. C 492)

Endometrial cancer was first diagnosed and histologically confirmed for the proband at age 69. According to the questionnaire, blood-relatives on the mother's side had endometrial cancer in two generations of the family tree. The proband's mother had endometrial cancer at age 64 and two mother's sisters had endometrial cancer diagnosed before age 50. Endometrial cancer was also diagnosed before age 50 for the proband's sister (age 49). Mean age at disease onset was 58 years. The proband's father had lung cancer after the age of 30. Gene mutations were determined for the proband. Mutations were found in two genes – *MSH6* and *MLH1*. Immunohistochemical examination was not performed because paraffin embedded tissues with healthy and cancer tissues were not available. The proband's both sisters were invited to a consultation after receiving the results of the genetic examinations. Both sisters were given a consultation and offered molecular genetic testing. One sister had no gene mutations. The other sister had a mutation in the *MSH6* gene (see Figure 2.5).

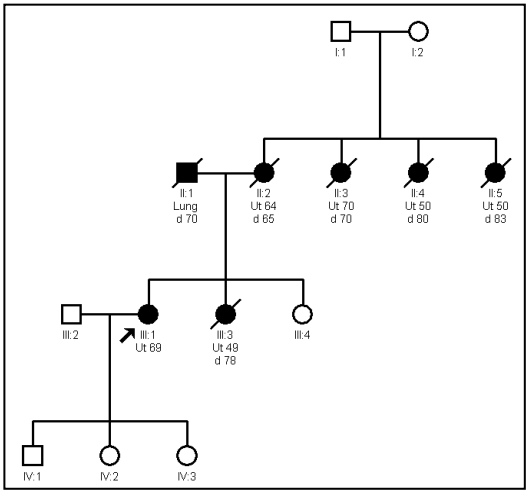

Figure 2.5. Patient R (ID No. C 492)

2nd case – patient R (ID No. C 458)

Endometrial cancer was first diagnosed and histologically confirmed for the proband at age 67. The patient's mother had uterine cancer before age 40 as the mother had died aged 41. The patient's maternal grandmother had uterine cancer. The age at diagnosis is not known but the grandmother died aged 60. The approximate mean age at disease onset is 55 years. The patient had molecular examination of her blood sample. Gene mutations were found in the *MSH2*, *MSH6* and *MLH1* genes. Healthy and cancer tissue samples were examined immunohistochemically; negative protein expression in the *MSH2* and *MSH6* genes was not found (see Figure 2.6).

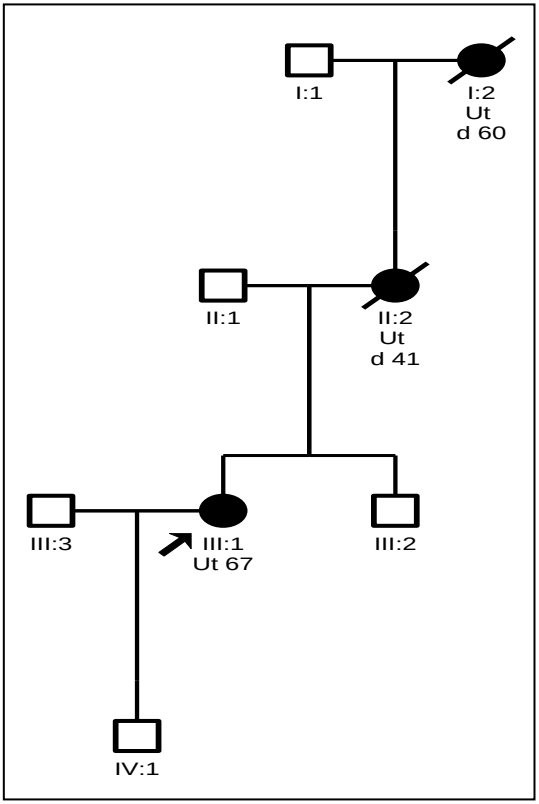

Figure 2.6. Patient R (ID No. C 458)

2.3.2. Family trees of an HNPCC-positive family

1st case – patient S (ID No. D 167)

Endometrial cancer was first diagnosed and histologically confirmed for the proband at age 61. The patient's mother had colorectal cancer at age 36, but she died at age 37. The patient's maternal grandmother also had colorectal cancer. The grandmother's age at disease onset is not known, but she died at age 47. The approximate mean age at disease onset is 47 years. The patient had her blood sample examined molecularly. A mutation was found in the *MLH1* gene but an additional blood sample was required to determine mutations in the *MSH2* and *MSH6* genes. As the patient had died after 12 months, it was not possible to obtain another blood sample. The healthy and cancer tissue samples for immunohistochemical examination were not available (see Figure 2.7).

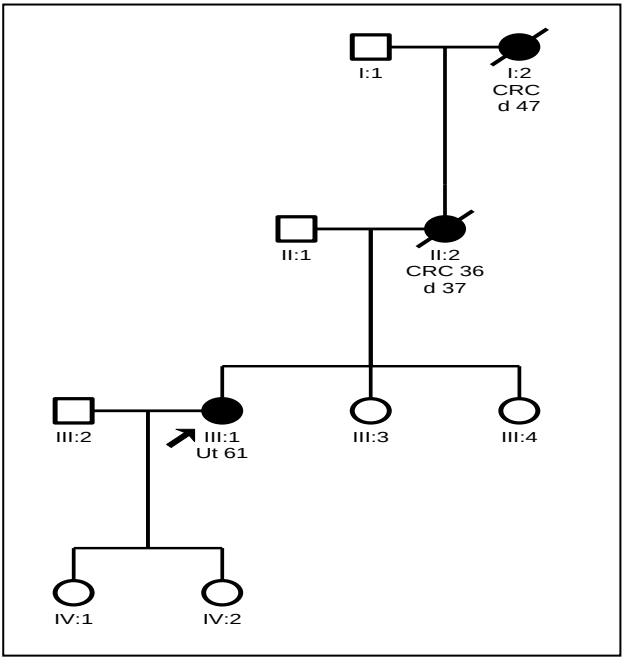

Figure 2.7. Patient S (ID No. D 167)

2nd case – patient A (ID No. F 004)

Endometrial cancer was first diagnosed and histologically confirmed for the patient at age 54. Patient's father had colorectal cancer and died aged 56. Patient's paternal grandmother had colorectal cancer at age 55. No mutations were found in the *MLH1*, *MSH2* and *MSH6* genes during molecular examination. Healthy and cancer tissue samples were not available for immunohistochemical examination (see Figure 2.8).

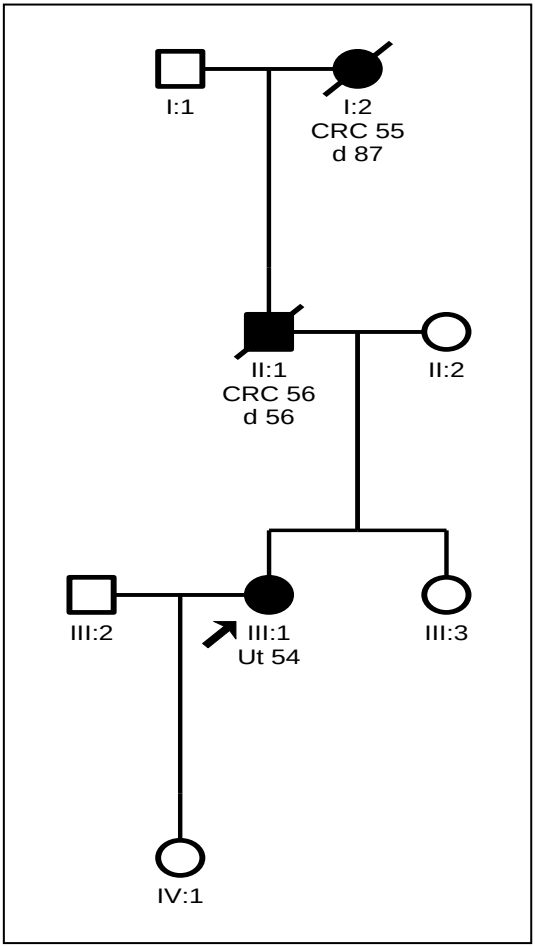

Figure 2.8. Patient A (ID No. F 004)

3. DISCUSSION

Several studies on endometrial cancer exist according to literature (Lynch et al., 1966; Hakala et al., 1991; Boltenberg et al., 1990). Lynch et al. (1966) indicate a study in which for 154 probands with endometrial cancer, similar symptoms were found in their first-degree relatives in 20 (13.0%) cases. The authors of another study indicated the connection of 4% of cases with HEC in families (mothers and sisters) by collecting oncological family histories for 51 patients with endometrial cancer (Boltenberg et al., 1990). In a similar study another group of authors distinguished patients with HEC in 8% of cases (Ollikainen et al., 2005).

This study included 704 consecutive patients with first diagnosed EC and, according to the diagnostic criteria, EC had been found for first-degree relatives in 19 (2.7%) families. The results were slightly different from the data available in literature which could be explained by having a much larger number of patients corresponding with the diagnostic criteria participating in the study, thus promoting higher credibility, i.e. the number of HEC patients was proportionally 2–3 times lower.

Longitudinal studies on the HNPCC syndrome have linked it with HEC, indicating that endometrial cancer is the second most common localization in these families after colorectal cancer. According to several authors, the risk of becoming afflicted with endometrial cancer is estimated between 40 and 60% in the framework of this syndrome (Aarnio et al., 1999; Dunlop et al., 1997). Our study included 704 patients with first diagnosed endometrial cancer over a four year period to determine how often, according to the diagnostic criteria, HEC occurs in families if in multiple generations several female blood relatives have had endometrial cancer as well as how often HEC occurs in the

framework of the HNPCC syndrome. The study results described in literature overall indicate an autosomal dominant heredity in families for patients with endometrial cancer (Lynch et al., 1966; Boltenberg et al., 1990; Ollikainen et al., 2005). According to our study, there are genetic mechanisms which possess hereditary tendencies.

In our study the existence of site-specific HEC as a separate genetic model coincides with similar data in the works of other authors (Boltenberg et al., 1990; Ollikainen et al., 2005). In the other group with the same genetic model there are families in which one of first-degree relatives has had colorectal cancer, endometrial cancer, ovarian cancer, according to the Amsterdam I or Amsterdam II criteria. A study is described where typical characteristics in the case of HNPCC have been found in 9/326 (3.1%) patients (Prat et al., 2007). According to literature, colorectal cancer is found in 1–5% cases in families with the HNPCC syndrome (Mecklin, 1987; Suomi et al., 1995). In several studies with patients with Lynch syndrome, endometrial cancer was common in 1.8 to 2.1% cases (Goodfellow et al., 2003; Ollikainen et al., 2005; Hampel et al., 2006). HNPCC might be one of the most common hereditary diseases; its incidence is 1/200 and 1/2000 in the population (Kee and Collins, 1991). Endometrial cancer is the second most common localization in families with the HNPCC syndrome (Marx, 1991; Mecklin and Jarvinen, 1991; Watson and Lynch, 1993). Based on the data of the average endometrial cancer incidence in Latvia, more than a half of all the endometrial cancer cases in Latvia were included. The results indicate the incidence of HEC in families with a positive oncological family history – 10/704 (1.42%) cases of the HEC syndrome and 9/704 (1.28%) within the HNPCC syndrome.

Apart from the already known mutations of the MMR genes responsible for HEC, genes with low and medium penetrance have also been identified and they are most likely influenced by multiple factors (Spurdle et al., 2011; Long

et al., 2012; Delahanty et al., 2013). However, this particular study focuses on patients with Lynch syndrome. It is known that sporadic endometrial cancer is more commonly diagnosed at an earlier stage but in about 10–15% of cases the cause of death is metastases. An even greater risk for an early recurrent tumour or metastases is for patients with Lynch syndrome. British and Danish scientists have done a study with 269 women from families with Lynch syndrome in connection with a survival study programme. The programme plans that women from these families where cancer symptoms had not been recognized to date would undergo ultrasound examinations. Two women had changes 6–24 months after an ultrasound examination. Endometrial cancer at an early stage was discovered by performing fractional abrasion of the uterus during normal ultrasound checkup (Dove et al., 2002).

In another study in the Netherlands, by performing vacuum aspiration for endometrial biopsy changes were found in three cases eight months after normal ultrasonographic examination (Rijcken et al., 2003). In a similar study in Finland 175 women with Lynch syndrome underwent transvaginal ultrasound. For six out of 11 patients examined cancer was detected by vacuum aspiration for endometrial biopsy (Renkonen-Sinsalo et al., 2007). In addition, American researchers did a study with 315 patients who had gene mutations. 61 women underwent complete risk-reducing hysterectomy. By continuing surveillance over ten years, it was discovered that in 33% of cases women who had not undergone risk-reducing surgery developed endometrial cancer (Schmeler et al., 2006).

Endometrial cancer is most commonly diagnosed from age 55 to age 75 in the general population (Lynch et al., 1994). The age of onset for all hereditary cancers is generally lower than for sporadic cancers (Lynch et al., 1966; Hakala et al., 1991; Benatti et al., 1993). However, there are studies in which HEC risk increases with age, such as after age 70 up to 71% (Hendriks et

al., 2004). According to several studies, endometrial cancer patients with the HNPCC syndrome under age 50 are mutation carriers in 4.9 to 9% cases (Berends et al., 2003; Lu et al., 2005; Hampel et al., 2006). There is a study in which its authors (Prat et al., 2007) have described patients with endometrial cancer without distinguishing the 50-year-old mark but noting the mean age at disease onset to be 61.8 and 65.3 years. However, in another study a different age of onset was indicated at 48.3 years with 26 patients involved in the study (Hakala et al., 1991). Overall results show that a higher risk of developing endometrial cancer, including HEC, is during the menopausal and post-menopausal period. A group of authors determined the median age as less than 55 years by analysing data from 13 European countries, taking the endometrial cancer risk factors into account (Bray et al., 2005).

This study shows that for the hereditary group endometrial cancer was diagnosed in stage I in 15/19 (78.9%) cases while 1/19 (5.3%) patients had stage II. Compared with the sporadic group, HEC patients were more often diagnosed in stage I than sporadic endometrial cancer patients – 419 (61.2%). On the contrary, there were three (15.8%) HEC patients whose stage was not specified which is proportionally almost twice as many as among the sporadic endometrial cancer patients – 60 (8.7%). If this is compared to some of the previously mentioned studies, it can be seen that in the HEC group the number of cases in stage I, which is 12 (60%), has a lower frequency compared to the 343 (77.6%) cases in the sporadic group, whereas in stage III the indicators are higher in the hereditary group at four (20%) cases contrasted with the control group – 24 (5.4%) (Hakala, 1991).

In relation to our results, it could be explained by our research having more patients (704) and wider age intervals. In this study most patients (434/704) were diagnosed with endometrial cancer in stage I. Oncological family history was collected according to the previously described diagnostic

criteria but not all of the patients knew the possible oncological diagnosis of their first- or second-degree relatives precisely. As stage I was more often diagnosed in younger patients, family history could be collected with higher precision.

If the results are compared with other gynaecological cancer localizations and breast cancer during the period of our study from 2006 to 2009 for patients who were treated at the Latvian Oncology Centre, they are as follows: in stage I ovarian cancer was found in 17.8% of cases, cervical cancer – 36.8% of cases, vulvar cancer – 14.5% of cases and breast cancer – 26.9% of cases (Centre of Health Economics of Latvia, 2009). Patients with endometrial cancer have a rather early diagnosis which can be explained by the fact that after the first symptom, i.e. bleeding from the reproductive tract women seek gynaecological help and pay attention to oncological diseases in the family, including endometrial cancer for close female relatives.

The number of Grade 1 and Grade 2 patients in the hereditary group was slightly higher than the sporadic group but overall the data were similar. The results did not differ much from the research of other authors (Hakala et al., 1991; Berends et al., 2003). Overall the results indicate there is not a significant difference in the stage of disease and the degree of differentiation in the HEC group.

It is possible to assess cancer process and the efficacy of therapy from the survival rate of patients and the recurrence of cancer or metastases at different time periods after therapy. It is influenced by the stage of disease at diagnosis. Histological findings are also important in the cancer process development. One of the most important factors for patients with endometrial cancer is the stage of disease. The five-year survival rate for patients with endometrial cancer after surgical treatment in stage I is 85–95%, in stage II – 75%, in stage III – 50%, and in stage IV – 20% (Chiang, 2011). In most cases

recurrences or metastases were found two years after treatment. Literature shows that most endometrial cancer patients are at an early stage, while 10–15% of these patients died from recurrent cancer or metastases. During the first two years of this study (2006 and 2007) recurrences or metastases were found in various organs as well as another cancer localization for nearly a half of the hereditary group's patients – 9/19 (47.4%). This draws attention to an increased aggressiveness of hereditary cancers compared with other endometrial cancers in the population despite the endometrial cancer patients having a rather early diagnosis and undergoing radical treatment. As there are no data about similar endometrial cancer findings in scientific literature, it cannot be compared with other studies.

In this study hereditary cancers not only had a higher risk of recurring or metastasizing but also higher mortality rates caused by the progress of cancer, compared to the sporadic group in which patients' diagnosis is not related to possible gene mutations. Five-year survival has been analysed in literature according to a cancer data base in the Netherlands. Data with varying ages and disease stages have been compared for 50 patients with endometrial cancer and the HNPCC syndrome against 100 patients with sporadic endometrial cancer, indicating that there is not a significant difference in five-year survival between the two groups. In stage I five-year survival was 92% for hereditary patients and 91% for sporadic patients, whereas in stage III five-year survival was 72% for hereditary patients and 50% for sporadic patients (Boks et al., 2002). The results of this study indicate higher mortality among HEC patients which could mean higher aggressiveness at the beginning of the disease process. This is further reinforced by a higher proportion of HEC patients having recurrences and metastases compared with the sporadic group.

In the further data summarization period, adding data on the deceased patients up to 1 December 2013, significant changes were not observed. Both

groups had a similar increase in the number of deceased patients. As there were comparatively few patients in the HEC group, this was responsible for a rather high percentage of deceased patients. If the data up to April 2010 and further up to December 2013 are compared, then no significant changes can be observed over the course of several years. With a wider selection of patients in the HEC group small differences in survival could develop.

In families with Lynch syndrome and further development of colorectal cancer the frequency of mutations is 85–90% in the *MLH1* and *MSH2* genes and 10–15% in the *MSH6* genes (Goodfellow et al., 2003; Quehenberger et al., 2005). Mutations in the *MSH2* and *MSH6* genes are more common for patients with endometrial cancer in the framework of the HNPCC syndrome (Doll et al., 2008; Garg et al., 2009). Mutations in the *MSH6* gene are common in older (after age 55) endometrial cancer patients (Wagner et al., 2001). MSI for endometrial cancer patients is common in 75% of cases that came about in the case of the HNPCC syndrome (Matias-guiu et al., 2001; Prat et al., 2007).

According to literature, immunohistochemical examination is one of the applicable methods to diagnose HEC more precisely, while previously selecting patients according to the diagnostic criteria, mutation carriers and considering the age of patients (50–60 years of age) (Kwon et al., 2011).

The hypotheses put forward have been confirmed – the characterizing mutations of HEC in Latvia can be found in the *MLH1*, *MSH2* and *MSH6* genes.

4. CONCLUSIONS

1. Analysing the clinical characteristics in families with an oncological family history as well as families with first- and second-degree relatives unaffected by cancer, no significant survival, stage and degree of differentiation differences were observed between the hereditary group and the sporadic group.
2. The clinical prevalence of HEC for consecutive hospitalized EC patients is 2.7% (95% CI: 1.7–4.2%).
3. The predisposing mutations of the HEC syndrome in Latvia are located in the *MSH2* and *MSH6* genes.
4. The lack of gene protein expression of the *MSH2* and *MSH6* genes during immunohistochemical examination is not a sufficiently effective selection criterion to discover new *MSH2* and *MSH6* gene mutation carriers in a group of consecutively hospitalized endometrial cancer patients in Latvia.
5. The sporadic endometrial cancer group had better survival than the hereditary endometrial cancer group.

5. PRACTICAL RECOMMENDATIONS

1. Each endometrial cancer patient should have their oncological family history collected by the general practitioner and the gynaecologist.
2. Oncological family histories should be evaluated and families that correspond with the diagnostic criteria of HEC (Amsterdam criteria) should be identified.
3. Families that correspond with the diagnostic criteria of HEC (Amsterdam criteria) should be given appropriate prophylactic recommendations.
4. In families that correspond with the diagnostic criteria of HEC (Amsterdam criteria), family members of the afflicted patient should be offered full examination of the *MSH6*, *MSH2* and *MLH1* genes.
5. In families with confirmed *MSH6*, *MSH2* or *MLH1* gene mutations, healthy blood relatives should be offered mutation screening and all healthy and afflicted mutation carriers should receive prophylactic treatment.
6. Gynaecological and ultrasound examinations are recommended once per year for blood relatives with proven *MSH2*, *MSH6* or *MLH1* gene mutations. In case of endometrial hyperplasia discovered during an ultrasound scan, the endometrium should be examined morphologically.
7. After the results of the examinations the risk of developing endometrial cancer should be explained to the person and radical hysterectomy offered.
8. People from families with confirmed site-specific HEC gene mutations who did not have any mutations discovered during screening should not take any prophylactic measures.

9. Immunohistochemical examination of the protein expression of the *MSH2* and *MSH6* genes followed by a sequencing of these genes is recommended for families with the HEC syndrome and HNPCC syndrome with tumour cell aggregation.

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