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THE IMPORTANCE OF CYCLIN D1, P53, CD56 ANTIGEN EXPRESSION IN PLASMA CELLS AND ITS CORRELATION WITH SEROLOGICAL AND CLINICAL PARAMETERS IN PATIENTS WITH MULTIPLE MYELOMA

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CD56, p53, and Cyclin D1 detection in plasma cells (PC) can help to predict prognosis of multiple myeloma (MM). Clinical and biochemical prognostic parameters were analysed in a group of 122 patients with primary diagnosed MM in the period 2011–2015. Bone marrow biopsies were analysed with Cyclin D1, p53, CD56 antibodies. Statistical analysis was performed using Microsoft Excel 2010 and Graph Pad Prism 5. Lack of CD56 expression and p53-positivity were significantly correlated with a low glomerular filtration rate (GFR), low platelet count and haemoglobin level, as well as with high serum creatinine levels. Patients with Cyclin D1 expression in PC had a significantly higher serum calcium level and more common osteolytic lesion in bones. CD56-negative as well as p53, Cyclin D1-positive groups had advanced Salmon–Durie MM stages by and significantly higher ß2-microglobulin. Expression of p53, Cyclin D1 and lack of CD56 antigen in PC are negative predictive factors in cases of MM, as these patients were diagnosed as having late Salmon–Durie stage and higher ß2-microglobulin level. Expression of p53 and lack of CD56 antigen in PC is associated with an increased creatinine level in blood and decreased GFR; therefore, these are criteria for chronic renal failure progression and poorer prognosis of MM.

Key words: *multiple myeloma, Cyclin D1, p53, CD56.*

INTRODUCTION

Multiple myeloma (MM) is the most common malignancy of plasma cells and accounts for about 1% of all malignant diseases and 10% of haematological malignancies (Lokhorst *et al*., 2002). 60–100 MM cases are diagnosed in Latvia yearly (Pildava, 2012).

Strong CD56-positivity in plasma cells (PC) of some patients with MM, in contrast to normal plasma cells that are mostly CD56-negative in others with MM, has been known since the 1990. However, recent studies of CD56 expression are contradictory. Aberrant CD56 expression has been demonstrated in 70% to 80% of MM cases at diagnosis (Harada *et al*., 1993; Harrington *et al*., 2009). Absence of CD56 expression in MM patients is associated with more aggressive disease and shorter overall survival (Rawstron *et al*., 1999; Hundemer *et al*., 2007; Sahara *et al*., 2009). Some studies have demonstrated a correlation between CD56-negative myeloma and bone lesions (Chang *et al*., 2006). Other authors have not found a significant correlation between CD56 expression and clinical parameters and overall survival (Dunphy *et al*., 2007).

Cyclin D1 is a product of the *CCND*1 gene and in association with cyclin-dependent kinases controls proliferation of

eukaryotic cells at specific points in G1, S, G2 phases. (Hitomi *et al*., 1999; Alt *et al*., 2000; De Falco *et al*., 2004; Padhi *et al*., 2013). Aberrant expression of Cyclin D1 in MM is associated with genetic abnormalities like translocation t (11;14)(q13;q32), polysomy for chromosome 11, amplification of CCND1 and other genetic abnormalities; this feature is associated with longer overall survival (Hitomi *et al*., 1999; Cook *et al*., 2006; Padhi *et al*., 2013). Immunohistochemically detected Cyclin D1 protein expression in myeloma cells has been reported in 24–50% cases; some researchers consider that it might provide clinically useful prognostic information (Markovic *et al*., 2004; Cook *et al*., 2006; Padhi *et al*., 2013).

Athanasiou *et al*. described a correlation between Cyclin D1 overexpression in MM and higher histological grade with favourable prognosis. These patients had a significantly lower level of haemoglobin (Athanasiou *et al*., 2001). However, there are studies that have not demonstrated any correlation between Cyclin D1 expression and clinical prognostic parameters (Markovic *et al*., 2004; Dunphy *et al*., 2007; Padhi *et al*., 2013).

p53 protein is a TP53 gene product, which has a crucial role in cell death and can be observed when cells undergo apoptosis in response to oncogenic stimuli. Normally, the cell cycle of cells with damaged DNA is arrested at G_1-S checkpoint until the damage is repaired, but cells that lack p53 or contain a mutant form are not arrested at G_1. Loss or mutation of TP53 is probably the most common single genetic change in malignancies when cells do not undergo apoptosis and so escape the control. Tumours that have not lost TP53 very often have mutated versions of it (Strachan *et al*., 1999; Soussi *et al*., 2010). MM patients with aberrant p53 nuclear expression usually are diagnosed in advanced clinical and histological stages, as well as have significantly shorter overall survival than patients without this abnormality. del(17p13) (TP53) and p53 immunohistochemical expression are strongly correlated (Chang *et al*., 2007; Souss *et al*., 2010; Chen *et al*., 2012).

Of course, overall survival of MM patients depends not only on the stage of MM, but also on different methods of chemotherapy (Soverini *et al*., 2003; Markovic *et al*., 2004; Lonial *et al*., 2016) and concomitant diseases. Some clinical and serological findings like osteolitic changes, glomerular filtration rate (GFR), level of ß2-microglobulin, M-protein, haemoglobin, calcium, albumin, creatinine etc. are useful for detection of MM stage and prognosis (Sailer *et al*., 1995; Goldschmidt, *et al*., 2001; Greipp *et al*., 2005). From 2005, the International Staging System for Multiple Myeloma has been used for MM staging, and the Salmon–Durie clinical staging system has been applied accordingly for the Classification of Multiple Myeloma (Greipp *et al*., 2005).

The aim of our study was to evaluate CD56, Cyclin D1, p53 expression in bone marrow tissue and its correlation with clinical and biochemical prognostic parameters of MM cases.

Our study is the first in European and Baltic countries that is based on the analyses of 122 patients. We found statistical correlation between more than 10 clinical and biochemical markers and expression of CD 56, p53, and Cyclin D1 in myeloma cells of bone morrow. These data will be used as predictive indicators of progression of multiple myeloma.

MATERIALS AND METHODS

Study population. Bone marrow biopsies of 122 patients from Rīga East Clinical University Hospital's Haematology Centre with MM diagnosed between 2011 and 2015 were enrolled in the study. Our study was designed in conformation to the Helsinki Declaration. The study protocol was approved by the Committee of Ethics, Rīga Stradiņš University.

Immunohistochemistry. Bone marrow biopsies were fixed in 10% neutral (pH = 7) buffered formalin solution, decalcified in "MicroDecfast" decalcification solution (Diapath, Italy), processed in "Sakura Tissue-Tek VIP 5" vacuum infiltration tissue processor and embedded in paraffin. All bone marrow biopsies were stained with routine haematoxylin and eosin, Periodic acid-Schiff (PAS), Giemsa stain and Gordon and Sweet's reticulin silver staining method using standard protocols (Bio-optica, Italy).

Immunohistochemical antigen expression was determined by a standard polymer based visualisation system (EnVision method by Dako/Agilen, Denmark/U.S.A.).

4–5-µm thick formalin fixed paraffin-embedded bone marrow biopsies were placed on adhesive positively charged slides. After de-waxing and rehydration, through alcohols to water, the slides were incubated with 3% H_20_2 for 10 minutes to inhibit endogenous peroxidase activity. The microwave-based antigen retrieval was performed in a freshly prepared 0.01 mol/l sodium citrate buffer (pH 6.0) solution at 750 W for 3 cycles, 10 minutes each.

Samples were immunostained using primary antibodies: Cyclin D1 (Clone-SP4, Ready-to-Use, Dako), p53 (Clone-Do-7, Ready-to-Use, Dako) and CD56 (Clone-123C3, at a 1 : 50 dilution, Dako) and_3.3?-diaminobenzidine-tetrahydrochloride dihydrate (DAB) as the chromogen (DAKO, Denmark). Slides were counterstained with Mayer's haematoxylin, dehydrated in alcohol, cleared in xylene and cover slipped.

Cyclin D1, p53, and CD56 expression in cytoplasm of myeloma cells was considered positive if more than 10% cells were stained with strong positive reaction, but considered negative if less than 10% of the cells were stained. We evaluated correlation between histological findings and patients' clinical data — gender, age, osteolytic lesion, laboratory data and stage by Classification of Multiple Myeloma according to the Salmon–Durie clinical staging system and the International Staging System (Durie and Salmon, 1975; Greipp *et al*., 2005) for MM.

Surgery material of five patients with nonspecific spondylitis was used as control. These samples were characterised as having prominent polyclonal plasmocytosis without atypical changes.

All slides were examined and photographed with a Leica Microscope (Leitz, Wetzlar, Germany).

Clinical-laboratory data. Normal levels of the studied clinical-laboratory parameters are shown in brackets: ß2-microglobulin (0–3 mg/l), albumin (34–52 g/l); haemoglobin (male: 126–175 g/l female 118–161 g/l, platelet count (150–410 × 10^9l), glomerular filtration rate-GFR (90 ml/min/1.73 m^2), creatinine (male: 30–106 µmol/l, female: 30–80 µmol/l); calcium level (2.2–2.6 mmol/l), M-protein.

Statistical analysis. Statistical analysis was performed using Microsoft Excel 2010 and Graph Pad Prism 5 software. The results were expressed as mean (M) ± standard deviation (SD) or percentage and range. Normality was assessed using the D'Agostino-Pearson omnibus normality test.

Correlation between histological, clinical and laboratory parametric data were determined by Pearson's or Spearman's tests. The Student's unpaired t-test or Mann-Whitney U test was used for comparing results between groups; differences were considered statistically significant at $p < 0.05$.

RESULTS

Distribution of the patients according to the Salmon–Durie clinical staging system was: I – 20% (n = 24); II – 45% (n = 55), III – 35% (n = 43). The patient mean age was 65 ± 10.81 years (range 36–81). The male to female ratio was 1: 1.3. Mean bone marrow cellularity in the whole group was 57.87% ± 20.64% (range 15–95%). Mean percentage of atypical plasma cells in BM biopsies was 52.14% ± 21.1% (range 15–90).

CD56 antigen expression in plasma cells. CD56 positive plasma cells (Fig. 1) were found in 95 (78%) cases. Significant positive correlation (Table 1) was shown by the Spearman test between CD56-positive expression and glomerular filtration rate, platelet count and haemoglobin level, but significant negative correlation was observed between CD56 expression and ß2-microglobulin, serum creatinine levels and Salmon–Durie clinical stage. Our results showed that the patient group with CD56-negative plasma cells had a significantly lower haemoglobin level, GFR (Fig. 2) and also lower platelet count, in comparison to the CD56-positive group. CD56-negative patients were predominately at an advanced Salmon–Durie disease stage and had signifi-

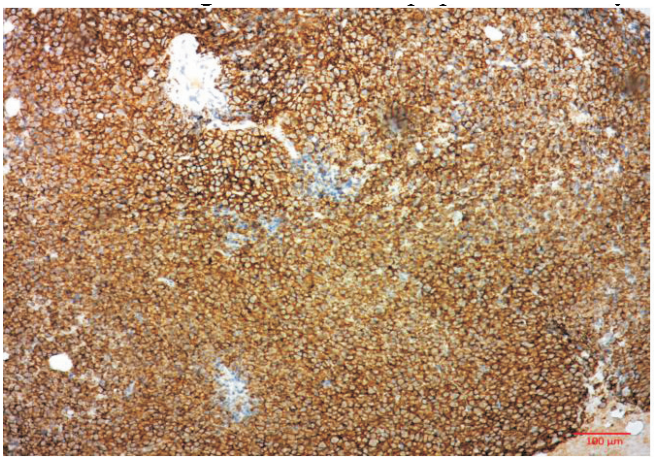

Fig. 1. Expression of CD56 antigen in a diffuse population of myeloma cells, 100×.

Table 1

CORRELATION OF CYCLIN D1, P53 AND CD56 EXPRESSION WITH CLINICAL AND LABORATORY DATA OF MULTIPLE MYELOMA PATIENTS

	CD56	Cyclin D1	p53
ß2-microglobulin, mg/dl	negative significant correlation (r = –0.22; p = 0.013)	positive significant correlation (r = +0.19; p = 0.033)	positive significant correlation (r = +0.27; p = 0.0025)
Albumin, g/l	ns	ns	ns
Haemoglobin, g/dl	positive significant correlation (r = +0.25; p = 0.005)	ns	negative significant correlation (r = -0.38; $p < 0.0001$)
Thrombocyte count, 10^9/l	positive significant correlation (r = +0.2; p = 0.031)	ns	negative significant correlation (r = –0.21; p = 0.023)
GFR, ml/min	positive significant correlation (r = +0.43, $p < 0.0001$)	ns	negative significant correlation (r = –0.21; p = 0.021)
Creatinine, µmol/l	negative significant correlation (r = –0.43; $p < 0.0001$)	ns	positive significant correlation (r = +0.21; p = 0.018)
Osteolytic lesions, %	ns	positive significant correlation (r = +0.29; p = 0.0014)	ns
Calcium, mmol/l	ns	positive significant correlation (r = +0.24; p = 0.009)	ns
M-protein, g/l	ns	ns	ns
Clinical stages by Salmon– Durie, n and %	negative significant correlation (r = –0.3; p = 0.0009)	positive significant correlation (r = +0.28; p = 0.002)	positive significant correlation (r = +0.41; $p < 0.0001$)

ns, not significant, $p > 0.05$)
GFR, glomerular filtration rate

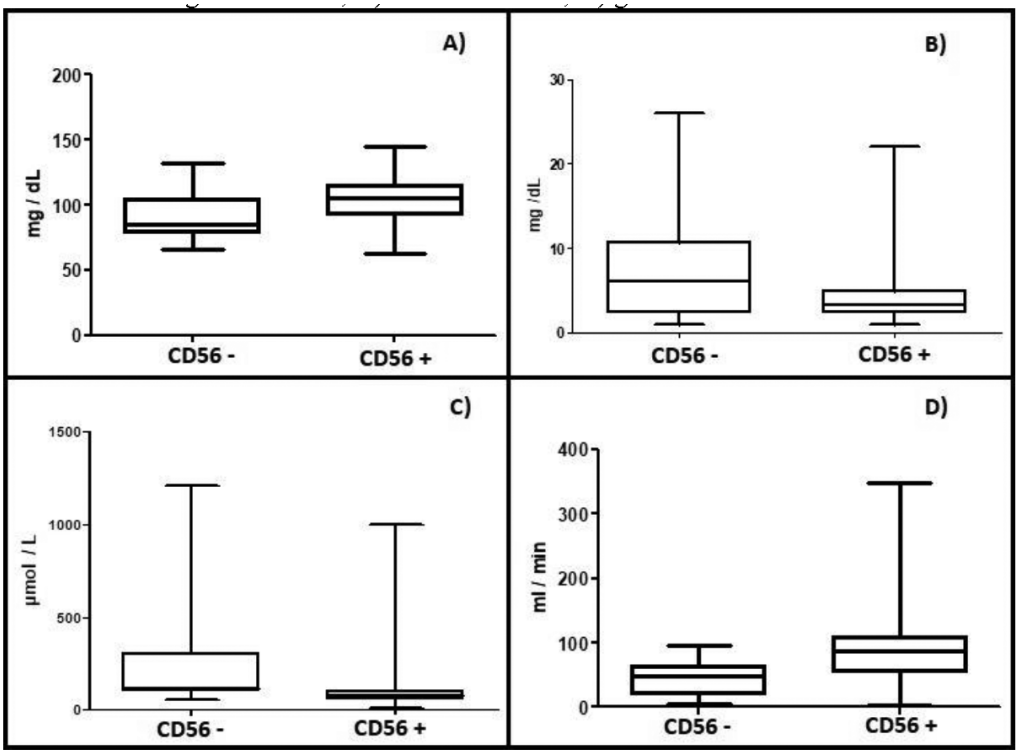

Fig. 2. CD56 antigen positive and negative groups: A) haemoglobin level; B) ß2-microglobulin level; C) creatinine level; D) glomerular filtration rate.

cantly higher ß2-microglobulin and serum creatinine (Fig. 2) levels, when compared to the CD56-positive group ($p < 0.05$). Patient gender, age, the level of LDH, albumin, CRP, calcium, and M-protein did not show any statistically significant differences between CD56-positive and negative groups (Table 2).

Cyclin D1 antigen expression in plasma cells. Cyclin D1 protein expression (Fig. 3) was detected in 62 (51%) cases. Positive correlation (Table 1) was observed between cyclin D1 expression and Salmon–Durie clinical stage, calcium, ß2-microglobulin level and osteolytic lesion in bones ($p < 0.05$). The patient group with positive Cyclin D1 expression in PCs (Table 3) had significantly higher ß2-microglobulin ($p = 0.034$) and calcium ($p = 0.0096$) levels, compared to those in the Cyclin D1 negative group (Fig. 4). The Cyclin D1 positive patient group had a more advanced Salmon–Durie stage and had significantly more pronounced bone osteolitic lesions, compared these parameters for the Cyclin D1 negative patient group ($p < 0.05$).

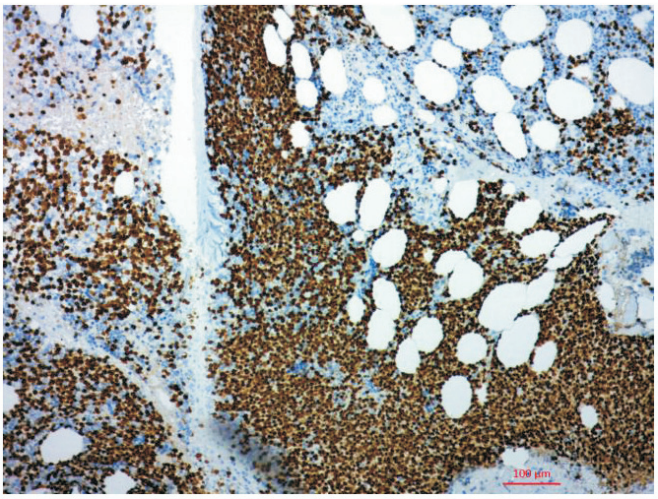

Fig. 3. Cyclin D1 positive plasma cells, 100×.

p53 antigen expression in plasma cells of MM patients. p53 protein was detected in 44 (36%) cases (Fig. 5). Significant positive correlation ($p < 0.05$) was observed between p53 protein expression in plasma cells (Table 1) and Salmon–Durie MM clinical stage, ß2-microglobulin and serum creatinine level. Furthermore, significant negative cor-

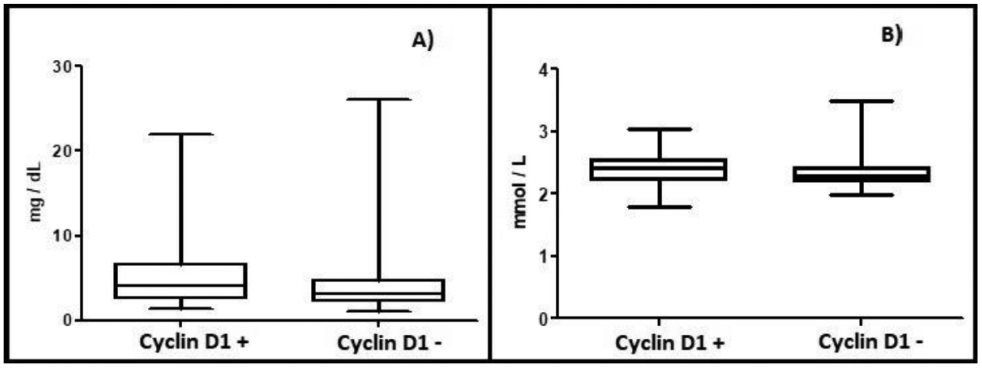

Fig. 4. Cyclin D1 positive and negative groups: A) ß2-microglobulin level; B) calcium level.

Table 2

CD56 EXPRESSION ACCORDING TO CLINICAL AND LABORATORY DATA OF MULTIPLE MYELOMA CASES*

	CD56 negative group n = 27	CD56 positive group n = 95	Statistical significance
Age, years	67.33 ± 7,1 (50–80) 95% CI 64.54–70,13	64.18 ± 10.63 (36–82) 95% CI 62.01–66.34	ns
Male / Female ratio	14 / 13	39 / 56	ns
ß2-microglobulin, mg/dl	7.81 ± 6.54 (1–26) 95% CI 5.17–10.45	4.11 ± 3.34 (1–22) 95% CI 3.43–4.79	$p = 0.0141$, Mann-Whitney U test
Albumin, g/l	40.21 ± 14.4 (26–97,3) 95% CI 34.52–45.91	40.77 ± 21.56 (23.4–238) 95% CI 36.38–45.16	ns
Haemoglobin, g/dl	93.33 ± 19.13 (66–132) 95% CI 85.77–100.9	104.3 ±17.6 (63–145) 95% CI 100.7–107.9	$p = 0.0059$, unpaired t test
Thrombocyte count, 10^9/l	184 ± 85.81 (43–352) 95% CI 150–218	228.4 ± 86.25 (26–475) 95% CI 210.8–246	$p = 0.0195$, unpaired t test
GFR, ml/min	45.82 ± 26.73 (6.2–95) 95% CI 35.25–56.4	87.36 ± 47.12 (3–348) 95% CI 77.76–97	$p < 0.0001$, Mann-Whitney U test
Creatinine, µmol/l	219.3 ± 239 (59–1211) 95% CI 124.7–314	101.1 ± 111.7 (9–1001) 95% CI 78.33–123.9	p 0.0001 Mann-Whitney U test
Osteolytic lesions, %	70.4%	62%	ns
Calcium, mmol/l	2.44 ± 0.35 (1.78–3.49) 95% CI 2.3–2.58	2,37 ± 0.25 (1,99–3.33) 95 CI 2.32–2.42	ns
M-protein, g/l	29.47 ± 20.99 (0–75.7) 95% CI 21.17–37.8	27.74 ± 17.53 (0–76.1) 95 CI 24.13–31.35	ns
Clinical stages by Salmon–Durie, n and %	I / II / III 1 / 10 / 16 4% / 37% / 59%	I / II / III 23 / 45 / 27 24% / 47% / 29%	$p = 0.0012$, Mann-Whitney U test
MM paraproteine Class: IgG / IgA / Bence Jones / non-secretory MM n and %	18 / 3 / 4/ 2 67% / 11% / 15% / 7%	62 / 20 / 10 / 3 65% / 21% / 11% / 3	ns

* mean ± SD; 95%. CI, confidence interval; ns, not significant, $p > 0.05$
MM, multiple myeloma

relation was observed between p53 expression and haemoglobin level, glomerular filtration rate and thrombocyte count. The p53-positive group (Table 4) had a more advanced Salmon–Durie MM stage ($p < 0.0001$), significantly higher ß2-microglobulin ($p = 0.029$), serum creatinine ($p = 0.0192$) levels, compared these parameters in the p53-negative group (Fig. 6).

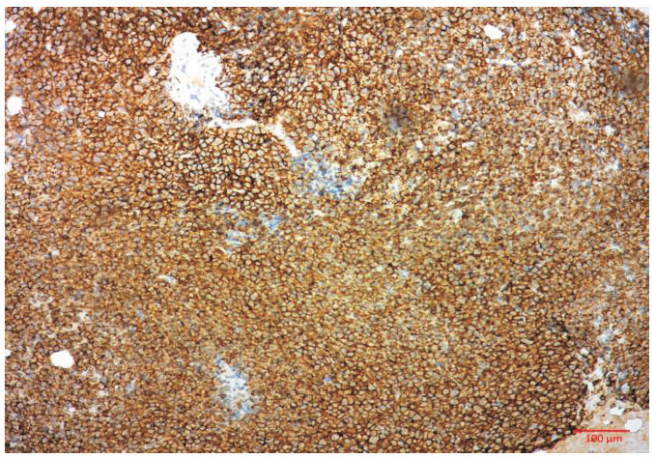

Fig. 5. p53 intranuclear expression in myeloma cells, 200×.

The p53-positive group had a significantly lower haemoglobin level ($p < 0.0001$) and platelet count ($p = 0.0188$) than in the p53-negative group. In addition, the p53-positive group had a lower GFR (Fig. 6), compared to that in the p53-negative group ($p = 0.0215$).

DISCUSSION

Histological examination combined with immunohistochemistry plays an important role in MM management. Besides being the gold standard for diagnosis, it provides prognostic information such as degree of bone marrow infil-

Table 3

CYCLIN D1 EXPRESSION ACCORDING TO CLINICAL AND LABORATORY DATA*

	Cyclin D1 positive group n = 62	Cyclin D1 negative group n = 60	Statistical significance
Age, years	64 ± 10.25 (36–81) 95% CI 61.4–66.6	65.78 ± 9.76 (43–82) 95% CI 63.26–68.31	ns
Male / Female ratio	26 / 36	27 / 33	ns
ß2-microglobulin, mg/dl	5.5 ± 4.58 (1.29–22) 95% CI 4.34–6.67	4.27 ± 4.3 (1–26) 95% CI 3.15–5.39	$p = 0.034$, unpaired t test
Albumin, g/l	36.68 ± 7.91 (24–58) 95% CI 36.67–40.69	42.68 ± 27.55 (23.4–238) 95% CI 35.56–49.79	ns
Haemoglobin, g/dl	99.6 ± 19.94 (63–142) 95% CI 94.53–104.7	104.2 ± 16.56 (70—145) 95% CI 99.94–108.5	ns
Thrombocyte count, 10^9/l	215.4 ± 88.29 (26–475) 95% CI 193–237.8	221.8 ± 87.87 (43–447) 95% CI 199.1–244.5	ns
GFR, mL / min	72.16 ± 40.54 (3–162) 95% CI 61.87–82.46	84.37 ± 51.88 (6–348) 95% CI 70.97–97.77	ns
Creatinine, µmol/l	150.2 ± 204.9 (42–1211) 95% CI 98.18–202.2	103.5 ± 74.27 (9–439) 95% CI 84.34–122.7	ns
Osteolytic lesions, %	48 77.4%	30 50%	$p = 0.0017$, Mann-Whitney U test
Calcium, mmol/l	2.43 ± 0.28 (1.78–3.04) 95% CI 2.36–2.5	2.34 ± 0.27 (1,99–3.49) 95 CI 2.27–2.41	$p = 0.0096$, Mann-Whitney U test
M-protein, g/l	29.35 ± 20.66 (0–76.1) 95% CI 24.06–34.64	26.86 ± 15.53 (0–57.8) 95 CI 22.82–30.91	ns
Clinical stages by Salmon–Durie, %	I / II / III 6 / 28 / 28 10% / 45% / 45%	I / II / III 18 / 27 / 15 30% / 45% / 25%	$p = 0.0025$, Mann-Whitney U test
MM paraproteine Class: IgG / IgA / Bence Jones / non-secretory MM n and %	43 / 7 / 8 / 4 69% / 11% / 13% / 7%	37 / 16 / 6 / 1 62% / 26% / 10% / 2%	ns

* mean ± SD; 95%. CI, confidence interval; ns, not significant, $p > 0.05$

tration, grade of tumour cell atypia and immunophenotype. Experimental treatment based on immunohistochemical findings was recently introduced for refractory MM, for example, by stimulation of p53-related apoptosis and antibody based treatment with anti-CD56 drugs (Soverini *et al*., 2003; Teoh *et al*., 2014; Lonial *et al*., 2016). The current tendency of individually tailored treatment implies that in the future, the immunophenotype of each MM patient will have to be taken into consideration, since genetic and immunohistochemical characteristics are very heterogeneous.

In the current study, we retrospectively analysed prognostic plasma cell antigenic biomarkers (Cyclin D1, CD56, p53) in 122 trephine bone marrow biopsies and evaluated their relation to serological, clinical and laboratory parameters in a large cohort of primary MM patients.

Several markers, like ß2-microglobulin, haemoglobin, and albumin levels have been proposed as prognostic factors in MM. It is also known that overall survival of MM patients is correlated with the creatinine level and GFR (Durie and Salmon, 1975; Greipp *et al*., 1988; Hartmut *et al*., 2001; Greipp *et al*., 2005). Since previous studies (Sailer *et al*., 1995; Greipp *et al*., 2005; Lonial *et al*., 2016) demonstrated prognostic significance of the immunohistochemically detected phenotype of tumour cells in MM, new data on relations between biochemical and histological prognostic markers could be of particular interest.

We detected an aberrant CD56 expression in myeloma cells in 78% of cases, using immunohistochemical method. According to literature data, patients that are CD56-negative (detected by flow cytometry) have poorer prognosis (Hague *et al*., 1994; Korsrneyer *et al*., 1992). Our CD56-positive

Table 4

CHARACTERISTICS OF P53 EXPRESSION ACCORDING TO CLINICAL AND LABORATORY DATA OF MULTIPLE MYELOMA PATIENTS*

	p53-positive group n = 44	p53-negative group n = 78	Statistical significance
Age, years	65.68 ± 11.67 (36–82) 95% CI 62.13–69.23	64.42 ± 9 (47–82) 95% CI 62.4–66.45	ns
Male / Female ratio	23 / 21	30 / 48	ns
ß2-microglobulin, mg/dl	5.84 ± 4.66 (1.29–21) 95% CI 4.42–7.255	4.37 ± 4.3 (1–26) 95% CI 3.39–5.34	$p = 0.029$ Mann-Whitney U test
Albumin, g/l	39.29 ± 11.92 (24–97.3) 95% CI 35.67–42.92	41.41 ± 23.59 (23.4–238) 95% CI 36.09–46.73	ns
Haemoglobin, g/dl	92.55 ± 17.13 (63–132) 95% CI 87.34–97.75	107.1 ±17.1 (68–145) 95% CI 103.3–111	$p < 0.0002$, unpaired t test
Thrombocyte count, 10^9/l	193.8 ± 87.65 (43–371) 95% CI 167.2–220.5	232.5 ± 85.3 (26–475) 95% CI 213.3–251.7	$p = 0.0188$, unpaired t test
GFR, ml/min	66.66 ± 44.51 (6–169) 95% CI 53.13–80.2	84.65 ± 46.9 (3–348) 95% CI 74.08–95.23	$p = 0.0215$, Mann-Whitney U test
Creatinine, µmol/l	164.5 ± 203.6 (48–1211) 95% CI 102.6–226.4	106.3 ± 118.1 (9–1001) 95% CI 79.62–132.9	$p = 0.0192$ Mann-Whitney U test
Osteolytic lesions, %	31 70.5%	47 60%	ns
Calcium, mmol/l	2.37 ± 0.32 (1.78–3.49) 95% CI 2.28–2.47	2.4 ± 0.25 (1.99–-3.33) 95 CI 2.34–2.45	ns
M-protein, g/l	29.72 ± 19.13 (0–75.7) 95% CI 23.83–35.61	27.24 ± 17.86 (0–76.1) 95 CI 23.18–31.29	ns
Clinical stages by Salmon–Durie, %	I / II / III 2 / 16 / 26 5% / 36% / 59%	I / II / III 22 / 39 / 17 28% / 50% / 22%	$p < 0.0001$, Mann-Whitney U test
MM paraproteine Class: IgG / IgA / Bence Jones / non-secretory MM n and %	27 / 9 / 6/ 2 61% / 20% / 14% / 5%	53 / 14 / 8 / 3 68% / 18% / 10% / 4%	ns

* mean ± SD; 95%. CI, confidence interval; ns, not significant, $p > 0.05$

group presented at late Salmon–Durie clinical stage, with a high ß2-microglobulin level, low haemoglobin level, low platelet count and renal insufficiency and was thus was associated with clinical and laboratory markers of poorer prognosis. Clinical follow-up with overall survival assessment seems to be required to resolve the contradiction.

Cyclin D1 is another immunochistochemical prognostic marker associated with genetic abnormalities. Cyclin D1 is necessary for the coordination of the cell cycle G1 / S transition, as cyclins act as regulators of cyclin dependent kinases and have a role in tumourogenesis. Some studies have shown it to have positive prognostic value (Markovic *et al*., 2004; Cook *et al*., 2006; Padhi *et al*., 2013). In our study we detected Cyclin D1 protein expression in 51% of cases, and it was associated with poorer prognostic indicators, like high ß2-microglobulin and calcium levels. In our analysed group of patients with Cyclin D1 overexpression there were more osteolytic lesions.

p53 was detected in 36% of examined cases using the immunohistochemical method. p53 overexpression was correlated with shorter overall survival. p53 plays a role in apoptosis (Chang *et al*., 2007; Souss *et al*., 2010; Chen *et al*., 2012). In our study, p53 expression in myeloma cells within a group of patients was also correlated with many prognostic clinical and laboratory findings, such as a high ß2-microglobulin level, late stage according to the Salmon–Durie classification, low haemoglobin level, platelet count and renal insufficiency indicated by a decreased GFR and elevated creatinine level.

We have found that overexpression of CD56, Cyclin D1 and p53 in myeloma plasma cells showed a statistically sig-

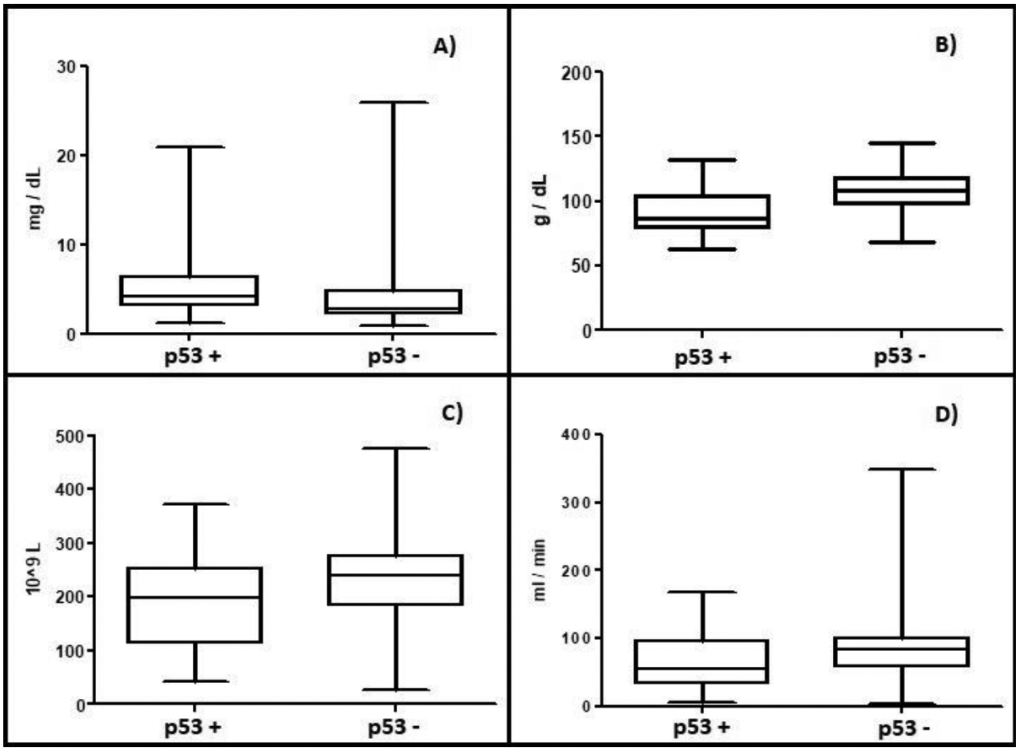

Fig. 6. p53 positive and negative groups: A) ß2-microglobulin level; B) haemoglobin level; C) thrombocyte count; D) glomerular filtration rate.

nificant correlation with various clinical and laboratory characteristics of MM. Positivity for p53 and Cyclin D1 and lack of CD56 antigen in myeloma plasma cells are negative predictive factors in MM, associated with an advanced Salmon–Durie stage and higher ß2-microglobulin levels. Chronic renal failure progression, which was also associated with poorer prognosis in MM patients was related to expression of p53 and lack of CD56 antigen in neoplastic plasma cells, as in these patients there was an increased creatinine level in blood and decreased GFR.

Many studies have evaluated Cyclin D1 as a factor of favourable prognosis and indicator of longer survival time in patients treated with standard and new generation medicine. There are studies about Cyclin D1 levels and it's correlations with clinical and laboratory data. In our study, Cyclin D1 overexpression was more typical for a graver Salmon–Durie stage and higher ß2-microglobulin level, thus showing progression of MM.

It is possible that patients with Cyclin D1 antigen expression in myeloma cells may have had improved responsiveness to chemotherapy treatment, which may explain the higher survival rate in previously published studies (Markovic *et al*., 2004; Cook *et al*., 2006; Padhi *et al*., 2013).

Statistical analysis of our study results showed that expression of Cyclin D1 was not correlated with the indicators of renal function and that these patients did not develop chronic renal failure, one of the most common complications of MM.

Thus, CD56, Cyclin D1 and p53 overexpression can be used for making more precise morphological staging of MM and can be applied as a predictive factor for indicating a more aggressive therapy regimen.

CONCLUSIONS

1. Expression of p53 and Cyclin D1, but lack of CD56 antigen in plasma cells, is a negative predictive factor in cases of MM, as late Salmon–Durie stage and higher ß2-microglobulin level are more common in these cases.

2. Expression of p53 and lack of CD56 antigen is associated with paraprotein hyperproduction in plasma cells, which in turn damages renal parenchyma and affects renal function and is associated with an increased creatinine level in blood and decreased GFR.

These correlations are criteria for chronic renal failure progression and poorer prognosis of multiple myeloma patients.

REFERENCES

Alt, J. R., Cleveland, J. L., Hannink, M., Diehl, J. A. (2000). Phosphorylation-dependent regulation of cyclin D1 nuclear export and cyclin D1-dependent cellular transformation. *Genes Devel.,* **14** (24), 3102–3114.

Athanasiou, E., Kaloutsi, V., Kotoula, V., Hytiroglou, P., Kostopoulos, I., Zervas, C., Kalogiannidis, P., Fassas, A., Christakis, J. I., Papadimitriou, C. S. (2001). Cyclin D1 Overexpression in Multiple Myeloma. *Amer. J. Clin. Pathol.,* **116**, 535–542.

Chang, H., Samiee, S., Yi. Q. L. (2006). Prognostic relevance of CD56 expression in multiple myeloma: A study including 107 cases treated with high-dose melphalan-based chemotherapy and autologous stem cell transplant. *Leukemia Lymphoma*, **47** (1), 43–47.

Chang, H., Yeung, J., Qi, C., Xu, W. (2007). Aberrant nuclear p53 protein expression detected by immunohistochemistry is associated with hemizygous P53 deletion and poor survival for multiple myeloma. *Brit. J. Haematol.*, **138** (3), 324–329.

Chen, M. H., Qi, C. X., Saha, M. N., Chang, H. (2012). p53 nuclear expression correlates with hemizygous TP53 deletion and predicts an adverse out-

come for patients with relapsed/refractory multiple myeloma treated with lenalidomide. *Amer. J. Clin. Pathol.*, **137** (2), 208–212.

Cook, J. R., Hsi, E. D., Worley, S., Tubbs, R. R., Hussein, M. (2006). Immunohistochemical analysis identifies two cyclin D1+ subsets of plasma cell myeloma, each associated with favorable survival. *Amer. J. Clin. Pathol.*, **125** (4), 615–624.

De Falco, M., Fedele, V., De Luca, L., Penta, R., Cottone, G., Cavallotti, I., Laforgia, V., De Luca, A. (2004). Evaluation of cyclin D1 expression and its subcellular distribution in mouse tissues. *J. Anat.*, **205** (5), 405–412.

Dunphy, C. H., Nies, M. K., Gabriel, D. A. (2007). Correlation of plasma cell percentages by CD138 immunohistochemistry, cyclin D1 status, and CD56 expression with clinical parameters and overall survival in plasma cell myeloma. *Appl. Immunohistochem. Mol. Morphol.*, **15** (3), 248–254.

Durie, B. G. M., Salmon, S. E. (1975). A clinical staging system for multiple myeloma. Correlation of measured myeloma cell mass with presenting clinical features, response to treatment, and survival. *Cancer*, **3** (36), 842–854.

Goldschmidt, H., Lannert, H., Bommer, J., Ho, A. D. (2001). Renal failure in multiple myeloma "The myeloma kidney": State of the art. *Saudi J. Kidney Dis. Transplant.,* **12** (2), 145–150.

Greipp, P. R., Katzmann, J. A., O'Fallon, W. M., Kyle, R. A. (1988). Value of beta 2-microglobulin level and plasma cell labeling indices as prognostic factors in patients with newly diagnosed myeloma. *Blood,* **72** (1), 219–223.

Greipp, P. R., San Miguel, J., Durie, B. G. M., Crowley, J. J., Barlogie, B., Bladé, J., Boccadoro, M., Child, J. A., Avet-Loiseau, H., Kyle, R. A., Lahuerta, J. J., Ludwig, H., Morgan, G., Powles, R., Shimizu, K., Shustik, C., Sonneveld, P., Tosi, P., Turesson, I., Westin, J. (2005). International staging system for multiple myeloma. *J. Clin. Oncol.,* **23** (15), 3412–3420.

Harada, H., Kawano, M. M., Huang, N., Harada, Y., Iwato, K., Tanabe, O., Tanaka, H., Sakai, A., Asaoku, H., Kuramoto, A. (1993). Phenotypic difference of normal plasma cells from mature myeloma cells. *Blood*, **15**, 81 (10), 2658–2663.

Harrington, A. M., Hari, P., Kroft, S. H. (2009). Utility of CD56 Immunohistochemical studies in follow-up of plasma cell myeloma. *Amer. J. Clin. Pathol.,* **132**, 60–66.

Hitomi, M., Stacey, D. W. (1999). Cellular ras and cyclin D1 are required during different cell cycle periods in cycling NIH 3T3 cells. *Mol. Cell. Biol.*, **19** (7), 4623–4632.

Hundemer, U., Klein, D. Hose, Raab, M. S., Cremer, F. W., Jauch, A., Benner, A., Heiss, C., Moos, M., Ho, A. D., Goldschmidt, H. (2007). Lack of CD56 expression on myeloma cells is not a marker for poor prognosis in patients treated by high-dose chemotherapy and is associated with translocation t(11;14). *Bone Marrow Transplant.*, **40**, 1033–1037.

Lokhorst, H. (2002). Clinical features and diagnostic criteria. In: Mehta, J., &Singhal, S. (eds.). *Myeloma*. Martin Dunitz Ltd., London, pp. 151–168.

Lonial, S., Durie, B., Palumbo, A., San-Miguel, J. (2016). Monoclonal antibodies in the treatment of multiple myeloma: Current status and future perspectives. *Leukemia*, **30** (3), 526–535.

Markovic, O., Marisavljevic, D., Cemerikic, V., Suvajdzic, N. , Milic, N., Colovic, M. (2004). Immunohistochemical analysis of cyclin D1 and p53 in multiple myeloma: Relationship to proliferative activity and prognostic significance. *Med. Oncol.*, **21** (1), 73–80.

Padhi, S., Varghese, R. G., Ramdas, A. (2013). Cyclin D1 expression in multiple myeloma by immunohistochemistry: Case series of 14 patients and literature review. *Ind. J. Med. Paediatr. Oncol.*, **34** (4), 283–291.

Pildava, S. (2012). Multiplā mieloma. *Doctus*, 16.07.2012. Available from: http://www.doctus.lv/2012/7/multipla-mieloma (last accessed 21.03.2016).

Rawstron, A., Barrans, S., Blythe, D., Davies, F., English, A., Pratt, G., Child, A., Morgan, G., Jack, A. (1999). Distribution of myeloma plasma cells in peripheral bloodand bone marrow correlates with CD56 expression. *Brit. J. Haematol.*, **104**, 138–143.

Sahara, N., Takeshita, A., Shigeno, K., Fujisawa, S., Takeshita, K., Naito, K., Ihara, M., Ono, T., Tamashima, S., Nara, K., Ohnishi, K., Ohno, R. (2002). Clinicopathological and prognostic characteristics of CD56-negative multiple myeloma. *Brit. J. Haematol.*, **117**, 882–885.

Sailer, M., Vykoupil, K. F., Peest, D., Coldewey, R., Deicher, H., Georgii, A. (1995). Prognostic relevance of a histologic classification system applied in bone marrow biopsies from patients with multiple myeloma: A histopathological evaluation of biopsies from 153 untreated patients. *Eur. J. Haematol.*, **54** (3), 137–146.

Soussi, T. (2010).The history of p53: A perfect example of the drawbacks of scientific paradigms. *EMBO Reports*, **11** (11), 822–826.

Soverini, S., Cavo, M., Cellini, C., Terragna, C., Zamagni, E., Ruggeri, D., Testoni, N., Tosi, P., De Vivo, A., Amabile, M., Grafone, T., Ottaviani, E., Giannini, B., Cangini, D., Bonifazi, F., Neri, A., Fabris, S., Tura, S., Baccarani, M., Martinelli, G. (2003). Cyclin D1 overexpression is a favorable prognostic variable for newly diagnosed multiple myeloma patients treated with high-dose chemotherapy and single or double autologous transplantation, *Blood*, **102** (5), 1588–1594.

Strachan, T., Read, A. P. (eds.) (1999). *Human Molecular Genetics. 2nd edn.* Wiley-Liss, New York, Chapter 18, p. 576.

Teoh, P. J., Chng, W. J. (2014). p53 abnormalities and potential therapeutic targeting in multiple myeloma. *BioMed Res. Int.*, 2014, 1–9.

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ANTIGĒNU P53, CIKLĪNA D1 UN CD56 EKSPRESIJA MIELOMAS ŠŪNĀS UN TO KORELĀCIJA AR SASLIMŠANAS KLĪNISKAJIEM UN LABORATORISKAJIEM RĀDĪTĀJIEM PACIENTIEM AR MULTIPLU MIELOMU

Multiplas mielomas (MM) šūnās vairāku onkogēno un aberanto marķieru p53, ciklīna D1 un CD56 ekspresijai ir zināma loma slimības prognozēšanā, lai gan dažkārt to nozīme literatūrā tiek vērtēta pretrunīgi. Pētījuma mērķis bija analizēt korelāciju starp p53, ciklīna D1 un CD56 ekspresiju mielomas šūnās ar saslimšanas klīniskajiem un laboratoriskajiem rādītājiem. Tika izvērtēti 122 slimnieku klīniski laboratoriskie dati un kaulu smadzeņu biopsijās imūnhistoķīmiski noteicām p53, ciklīna D1 un CD56 ekspresiju. Statistiskā analīze veikta ar *Microsoft Excel* 2010 un *Graph Pad Prism* 5. CD56 antigēna iztrūkums mielomas šūnās, bet ar p53 ekspresija tajās statistiski ticami korelēja ar samazinātu trombocītu skaitu, kā arī ar samazinātu hemoglobīna līmeni un paaugstinātu kreatinīna līmeni, bet ar pazeminātu glomerulu filtrācijas ātrumu, kas liecina par nieru mazspējas progresiju. Savukārt, ciklīna D1 ekspresija statistiski ticami bija saistīta ar paaugstinātu kalcija līmeni serumā un klīniski radioloģiskiem osteolītiskiem kaulu bojājumiem. MM slimniekiem ar CD56 antigēna iztrūkumu, un ar p53 un ciklīna D1 ekspresiju statistiski ticamas bija vēlīnākas saslimšanas stadijas pēc *Salmon–Durie* klasifikācijas un attiecīgi augstāks ß2-mikroglobulīna līmenis asinīs, kas norāda uz sliktāku prognozi saslimšanai.