VILNIUS UNIVERSITY

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THE VALUE OF PLASMA CELL IMMUNOPHENOTYPIC ANALYSIS ESTIMATING RESPONSE TO TREATMENT AND RISK OF MULTIPLE MYELOMA

Summary of the Doctoral Dissertation

Biomedical sciences, medicine (06 B)

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VILNIAUS UNIVERSITETAS
MEDICINOS FAKULTETAS

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PLAZMINIŲ LĄSTELIŲ IMUNOFENOTIPINĖS ANALIZĖS REIKŠMĖ VERTINANT MIELOMINĖS LIGOS RIZIKĄ IR ATSAKĄ Į GYDYMĄ

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List of abbreviations used in the text

BM	bone marrow
CPC	circulating plasma cells
EP	early progression
HD	healthy donor
LC	light chains
LM	light microscopy
MFC	multiparameter flow cytometry
MFI	mean fluorescence intensity
MM	multiple myeloma
OS	overall survival
PC	plasma cells nPC - normal plasma cells (CD19+/CD56-); aPC - aberrant plasma cells (CD19-/CD56+); dpPC - double positive plasma cells (CD19+/CD56+); dnPC - double negative plasma cells (CD19-/CD56-); mPC - malignant plasma cells; m(a)PC - malignant aberrant plasma cells (CD19-/CD56+); m(dn)PC – malignant double negative plasma cells (CD19-/CD56-);
RR	relapsed or refractory
TLC	total living cells
TTP	time to progression

1. INTRODUCTION

Multiple myeloma (MM) is a malignant disease of terminally differentiated B lymphocytes - plasma cells (PC). It is second most commonly diagnosed cancer of hematopoietic system and constitutes 0.8% of all cancers. Firts effective therapy for multiple myeloma was discovered only four decades ago by R. Alexanian and colleques. They introduced into clinical practise melphalan based chemotherapy, which was the mainstay of MM treatment for the next 30 years. Treatments for MM have expanded in the last decade. New effective treatment options such as high dose chemotherapy, new drug classes (immunomodulators, proteosome inhibitors) signifficantly improved MM outcomes and influenced clinical practise. New treatment paradigm raised many new clinicall challenges. Some of these issues are adressed in these thesis.

One of the main issue is MM risk reassessment in the era of new treatments. Traditional Durie-Salmon staging system was widely accepted and endured during the last 30 years, but during the last five years it was almost completely replaced by recently introduced International Staging System (ISS). However even new, ISS staging system is not comprehensivelly validated as universal risk assessment method. Risk determination in relapsed or refractory (RR) MM setting is even more uncertain. ISS seems to be of little value in RR MM patients. Currently several new risk assessment directions are under throughout investigation in MM. Cytogenetic abnormalities have already emerged as one of the most powerfull risk factor in MM. Immunophenotyping of plasma cells using multiparameter flow cytometry (MFC) provides several possibilities establishing the risk of MM. Quantification of circulating plasma cells (CPC) by MFC, immunophenotypic pecularities, proportion of residual normal bone marrow plasma cells was shown to be of independant prognostic significance in newly diagnosed MM patients. However there was some shortcomings in MFC methodology used in previous CPC‘s studies. Prognostic significance of CPC‘s was not evaluated in RR MM patients.In this study we employed novel MFC methodology for plasma cell subpopulation analysis. We analyzed bone marrow and peripheral blood plasma cells from healthy controls and compared it with data from MM patients for optimization of our MFC methodology separating normal and malignant plasma cell subpopulations. We also evaluated prognostic value of malignant CPC‘s (mCPC) detection in advanced MM patients.

Another important issue that emerged due to increased number of effective treatment options is the lack of reliable criteria for selection of the most effective, patient specific salvage treatment in RR MM setting. Early evaluation of response to treatment may partially overcome this drawback. Paraprotein analysis as a surogate marker of disease burden is the mainstay of standart response assessment criteria in MM. Given its long half-life, paraprotein response is evaluable only after two to three months from the start of therapy and is not suitable for early response assessment. Considering the finding that malignant CPC proportion is decreasing during effective treatment, we hypothetised, that being the part of malignant plasma cell clone, it could serve as a direct marker of disease burden. Supposing that malignant CPC‘s specifically reflects agrressive disease clone mass whis is responsible for disease progression, malignant CPC‘s kinetics may emerge as a reliable marker differentiating „non responding, non progressing“ disease, from disease at risk for early progression. In this study we for the first time evaluated

kinetics of malignant CPC‘s proportion in response to first chemotherapy cycle as a marker of refractoriness to given treatment, predicting early progression.

Aim of the study

To explore the value of plasma cells immunophenotypic analysis using multiparameter flow cytometry as a method for assessment of prognosis and early response to treatment in advanced multiple myeloma patients.

Objectives of the study

1. To explore simultaneous CD19, CD56 and CD45 marker expression on healthy donors peripheral blood and bone marrow plasma cells.

2. To determine quantitative and immunophenotypic characteristics of healthy donors plasma cell subpopulations aberrantly expressing CD19 and CD56 markers and compare it with malignant plasma cell population from multiple myeloma patients.

3. To establish immunophenotypic criteria for definition of malignant and normal circulating plasma cell subpopulations.

4. To explore prognostic value of pretreatment detection of circulating plasma cells in advanced multiple myeloma setting.

5. To evaluate kinetics of circulating plasma cell subpopulations in response to one chemotherapy cycle and establish its prognostic value.

6. To determine quantitative characteristics and prognostic value of multiple myeloma patients peripheral blood normal plasma cell subpopulation.

7. To evaluate prognostic value of pretreatment mBMPC/nBMPC ratio established by multiparameter flow cytometry in advanced myeloma patients.

Scientific novelty

We introduced into clinical practise novel flow cytometric methodology for evaluation of plasma cells and optimized immunophenotypic differentiation between normal and malignant plasma cell subpopulations. We explored simultaneous expression of CD19, CD56 and CD45 markers on normal plasma cells from healthy individuals and for the first time described quantitative and immunophenotypic peculiarities of immunophenotypically aberant (CD19-/CD56+) normal plasma cells. We created and explored new method for the assessment of early response to treatment based on kinetics pattern of CPC‘s during the first chemotherapy cycle. We showed, that our method was capable to identify patients refractory to given therapy and at risk for early progression, with better accuracy than the standart criteria. We find that detection of CPC‘s is assotiated with more aggressive disease biology and shortened time to disease progression (TTP) in RR MM patients. After some additional studies, this marker could

serve as an important determinant for treatment decisions. We also for the first time described quantitative characteristics of peripheral blood normal plasma cell subpopulation in multiple myeloma patients and evaluated its clinical significance. We found proportion of residual normal bone marrow plasma cells in RR MM patients of prognostic significance and proved immunophenotypic analysis of bone marrow samples to be more prognostically important than traditional light microscopy in RR MM patients.

Practical significance of the study

Data about immunophenotypically aberrant normal plasma cell subpopulation and our proposed flow cytometric methodology could help to prevent mistakes analyzing small malignant plasma cell subpopulations. We have found, that nonreduction of mCPC's in relapsed or refractory myeloma patients after the first cycle of therapy may be useful in identifying patients early who are resistant to the administered therapy. If our findings will be confirmed in larger studies, these patients may be candidates for immediate switch to alternative therapy preventing them from unfavourable sequela of disease progression.Cessation of expensive, but ineffective treatment will also result in significant economic benefits.

Defensive theses

1. Six colors, two tubes flow cytometric methodology used in this study could reliably differentiate peripheral blood normal and malignant plasma cells
2. In bone marrow of majority healthy individuals there is detectable plasma cell subpopulation carrying combined aberrant CD19 and CD56 markers expression
3. mCPC detection before treatment in RR MM patients is assotiated with clinically aggressive disease and shortened TTP
4. Establishment of mCPC kinetics pattern in response to one chemotherapy cycle could identify RR MM cases refractory to given treatment and at risk for early progression with better accuracy than standart response evaluation method
5. Evaluation of normal plasma cell subpopulation in RR MM patients using flow cytometry provides clinically important information

2. PATIENTS AND METHODS

2.1. Study population

Patients were prospectively included if they met the following inclusion criteria: were 18 years of age or older, had relapsed or refractory multiple myeloma according to EBMT criteria after at least one prior line of therapy and were scheduled to receive either a Bortezomib (B) containing regimen or VAD (vincristin, doxorubicin and dexamethasone). Clinical and laboratory data including age, sex, type of paraprotein, β2 microglobulin levels, time from diagnosis, the number of lines of therapy, previous high-dose therapy were recorded. All patients signed informed consent. The study was approved by the Lithuanian Bioethics Committee. Forty two adult patient with RR MM

were prospectively enrolled into the study. Median age was 59 years. Clinical and laboratory data are summarized in Table1.

Healthy bone marrow donors (HD), were used as a control population. Bone marrow analysis is a standart procedure for sibling donors. HD were included into study if they signed informed consent and had negative immunofixation to exclude any paraproteinaemia.11 HD met inclusion criteria during data collection period and were enrolled into study - five females and six males with the median age of 43 (range 19–74) years.

2.2. Course of the research

Malignant CPCs (mCPC) were evaluated within one week before the start of the first therapy cycle and then three weeks later, immediately before the second therapy cycle. We allocated patients according to mCPC kinetics pattern to one of three groups: patients with no detectable mCPCs in both pre and postchemotherapy samples (group I), patients with a decrease in mCPCs postchemotherapy as compared to mCPCs before chemotherapy (group II) and patients with no change or increase in mCPCs postchemotherapy as compared to mCPCs before chemotherapy (group III)

Full examination for EBMT response category establishment, including bone marrow analysis and radiological examination when required, were performed after I-st, 4-th cycle and after completion of treatment. Patients were evaluated for the signs of disease progression before each cycle during treatment period and every three months during follow up thereafter. Data about time to progression and overall survival of study patients were collected.

2.3. Flow cytometry

Peripheral blood and bone marrow samples were collected directly into K2 – EDTA Vacutainer tube (BD, Plymouth). All samples were prepared by erythrocyte lysed whole blood technique to have as close cell population ratios as possible to the primary samples. Ficoll gradient cell separation and concentration procedure was not used due to possible bias in rare cell population ratios. We employed both the usual cell surface marker staining and cytoplasmic immunoglobulin light chain detection using Fix/Perm (BD, San Jose) cell permeabilization solution. This was the method of choice for the light chain restriction studies since intracellular light chain expression in PC‘s is more readily detectable than cell surface expression. All samples were stained with isotypic controls, corresponding to the monoclonal antibody type used in the experiment to establish negative fluorescence signal threshold for each fluorochrome and to evaluate nonspecific binding. Assay for plasma cells was performed with two tubes stained with antibody combinations (FITC/PE/PerCP-Cy-5.5/APC/PE-Cy7/APC-Cy7): CD56/CD138/CD45/CD19/CD38/CD20 and cLambda/cKappa/CD138/CD19/CD38/CD56. All monoclonal antibodies were from BD, San Jose, except for CD19-APC and CD138-PE, which were from Pharmingen, Palo Alto. The choice of markers was in agreement with the European Myeloma Network (EMN) consensus recommendations. The data was acquired on 6 color multiparameter FACSCanto flow cytometer equipped with FACSDiVa v6.1.2 data acquisition and analysis software (BD, San Jose, CA). Certain data acquisition limits were established.

The maximum sample total living cells (TLC) count was set at 1 x 10^6 and data was collected until maximum cell count or the end of the aliquot was achieved. The antigenic profile of PC‘s was based on the presence of antigen expression, the pattern of antigen expression (homogeneous vs. heterogeneous) and the percentage of PC‘s showing the positive expression of a given antigen having in mind that aberrant and normal PC‘s should be separated by different expression of CD19, CD56 and CD45 markers on cell surface and the light chain restriction. Immunophenotype of plasma cells was identified by gating on a population of CD38/CD138 positive cells and backgating on both markers vs. light side scatter (SSC). Then the expression of CD19 and CD56 markers on this population was analyzed (Fig. 1). Thus four regions of PC populations were defined:

1 CD19+/CD56- -normal plasma cells (nPC);

2 CD19-/CD56+ - aberrant plasma cells (aPC);

3 CD19+/CD56+ - double positive plasma cells (dpPC);

4 CD19-/CD56- - double negative plasma cells (dnPC).

Malignant PC populations of MM patients, as defined by immunophenotypic abberancies and monoclonal LC expression, were labeled by letter „m“ (e.g. mCPC, mBMPC). Specific immunophenotype of malignant PC subpopulations, where important, we expressed by the combined labeling - m(a)BMPC (malignant CD19-/CD56+ BMPC), and m(dn)BMPC (malignant CD19-/CD56- BMPC).

All four PC subpopulations defined by CD19 and CD56 expression were analyzed for the expression of CD45, CD20 and intracytoplasmic light chains. Quantitative characteristics of plasma cell subpopulations were established based on the data acquired from the first tube. The second tube was used to confirm aCPC monoclonality. Monoclonality was defined by at least > 4:1 or < 1:3 kappa/lambda light chain restriction.

In order to achieve a better specificity, the definition of a cell population was set as more than 20 or 100 cells in peripheral blood and bone marrow, respectively. The assay sensitivity was at least 2×10^{-4} and depended on both the TLC number acquired and the event number in PC population gate. PC populations containing less events than defined minimal treshold were defined as „0“.

At least two fold difference in mCPC proportions between the first and the second assessment points was considered significant. Data is presented as percentage of TLC in the sample analyzed.

2.4. Statistical analysis

The major goals of the study were to assess the impact of the CPC variables on early progression (EP), overall survival (OS) and time to tumor progression (TTP). EP was defined as a disease progression according to EBMT criteria during the planed treatment periodo of 126 days (duration of six 21 day cycles). OS was defined as the duration between the date of entry into the study and death, with those alive censored at the last follow-up date. TTP was defined as the interval from entry into the study to disease progression. Survival was analyzed by Kaplan and Meier method. Differences between survival curves were evaluated using the log-rank test. Comparison among subgroups was performed using Wilcoxon signed ranks, Mann-Whitney, Kruskal-Wallis tests and Chi Square statistics. Receiver-operating characteristic (ROC) curve analysis was used to determine diagnostic test accuracy. Multivariate analysis was performed

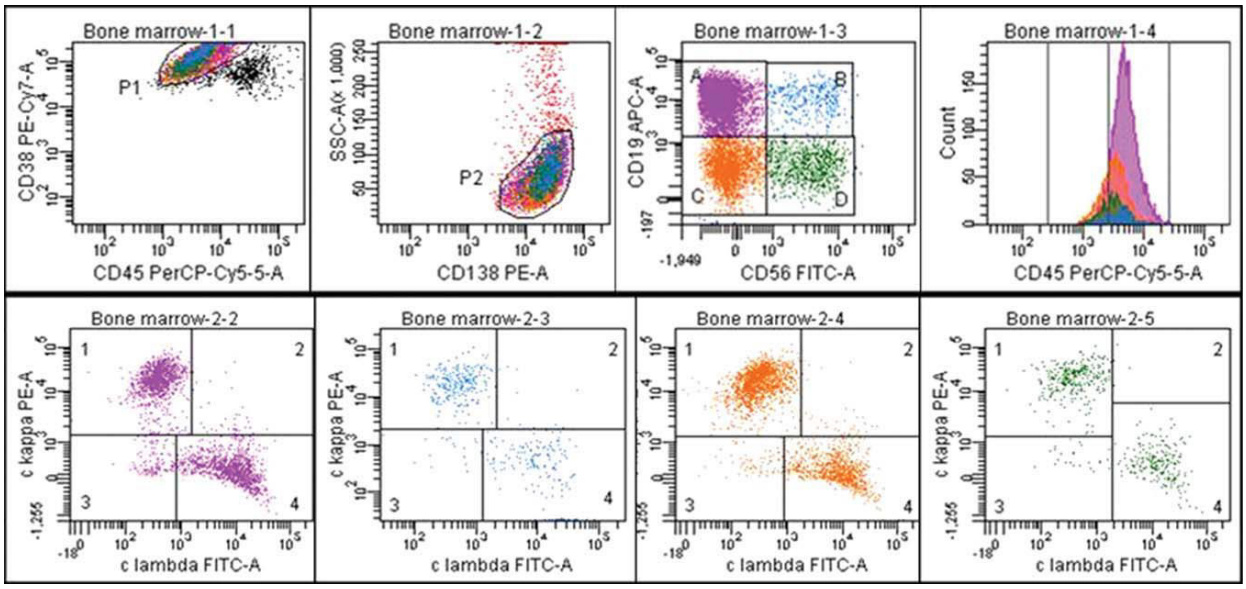
using logistic regression model for early progression (EP) and Cox proportional hazards model for TTP and OS. We used the classification table of the logistic regression model to define the best cutoff point for categorization of study patients according to mCPC kinetics. A two-tailed P value less than 0.05 was considered significant.

3. RESULTS

3.1. Healthy donors BMPC subpopulations

The median proportion of BM PCs in HDs detected using FC was 0.34% (0.1–0.84%). In CD19/56 dot plot, plasma cells distributed in four discrete subpopulations with clear separation between CD19 positive and negative cells and more gradual transition from CD56 negative to CD56 positive cells (Fig. 1).

Fig. 1 Immunophenotyping of bone marrow plasma cells in healthy donors.



Plasma cells were selected by gating on CD38/CD138 positive events (Bone Marrow views 1-1 and 1-2). These cells are tested for CD56 and CD19 expression, and four populations A (nBMPC), B (dpBMPC), C (dnBMPC), and D (aBMPC) are defined (Bone Marrow view 1-3). Bone Marrow view 1-4 shows CD45 expression on A, B, C, and D populations. Populations A, B, C, and D have polyclonal cytoplasmic kappa and lambda light chain expression regardless of CD56 and CD19 expression pattern in bone marrow of healthy donors as shown in Bone Marrow views from 2-2 to 2-4.

CD138+/CD38+/CD19+/CD56- cells (nBMPC) usually defined as immunophenotypically normal PCs was the largest BM PC subpopulation in all healthy donors and consisted of median 60.3% (37.3–72.3) of the total BM PCs.

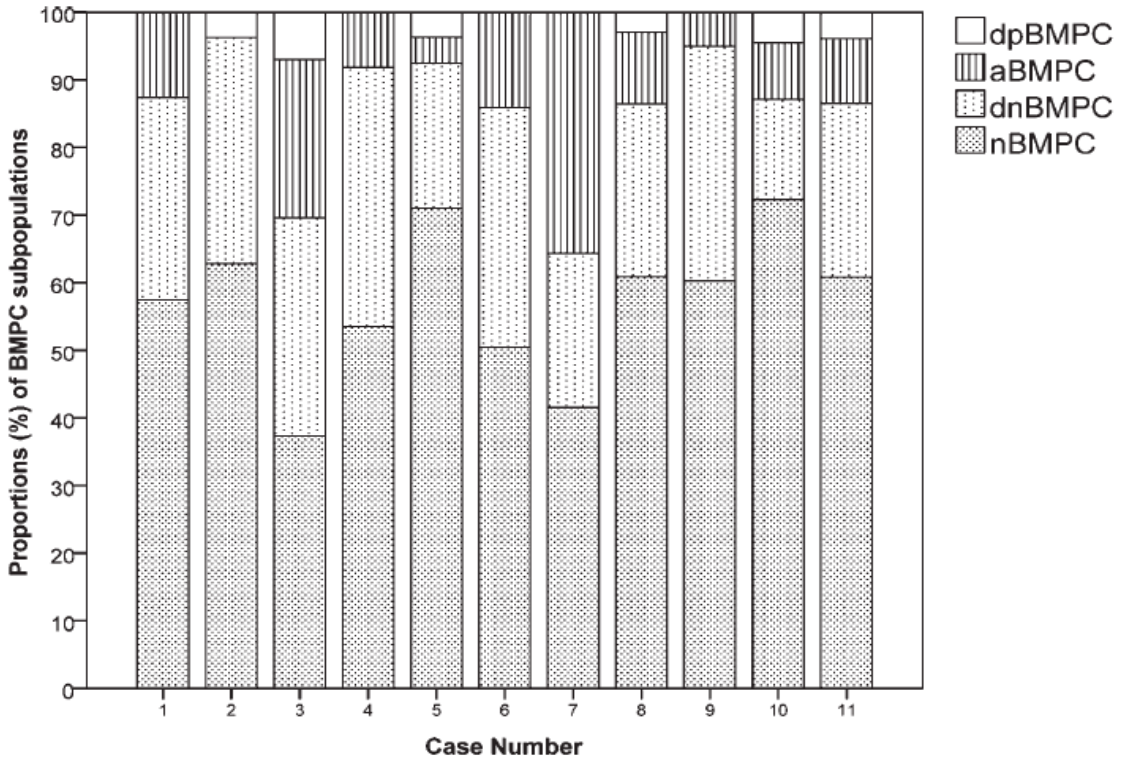
Using 100 cells treshold we were able to identify immunophenotypically aberrant BMPC subpopulations in all HD. CD19-/CD56+ (aBMPC) subpopulation exceeding 100 events (above the sensitivity level of 0.01%) were detected in 10 of 11 HDs'. The median proportion of aBMPC subpopulation was 9.6% (0–35.7%). The median number of detected events in aBMPC gate was 218 (70–983). In two HD, aBMPC population

exceeded 20% of the total BMPC population and could be considered positive by conventional immunophenotypic criteria.

CD19-/CD56- (dnBMPC) immunophenotype may be expressed by malignant PC clone in ~1/4 of MM cases. In our study dnBMPC subpopulation was the second largest after nBMPC having the median of 29.9% (14.8– 38.3%). In all HD‘s it exceeded 100 events and in 10 of 11 HD cases consisted of more than 20% of the total BM PCs.

We analyzed kappa and lambda LC expression on all BM PC subpopulations from HDs’ and found them to be polyclonal by kappa and lambda LC expression. Median values (ranges) of kappa/lambda ratios in HDs’ nBMPC, aBMPC, dnBMPC, dpBMPC were 1.3 (1–2.4), 1.8 (0.9–2.9), 1.9 (1.7–2.7), and 1.2 (0.9–1.4), respectively.

Fig. 2 Proportions of CD19/CD56 defined BMPC subpopulations in healthy donors



dpBMPC - double positive bone marrow plasma cells (CD19+/CD56+), dnBMPC - double negative bone marrow plasma cells (CD19-/CD56-), aBMPC - ‘‘aberrant’’ bone marrow plasma cells (CD19-/CD56+), nBMPC - ‘‘normal’’ bone marrow plasma cells (CD19+/CD56)

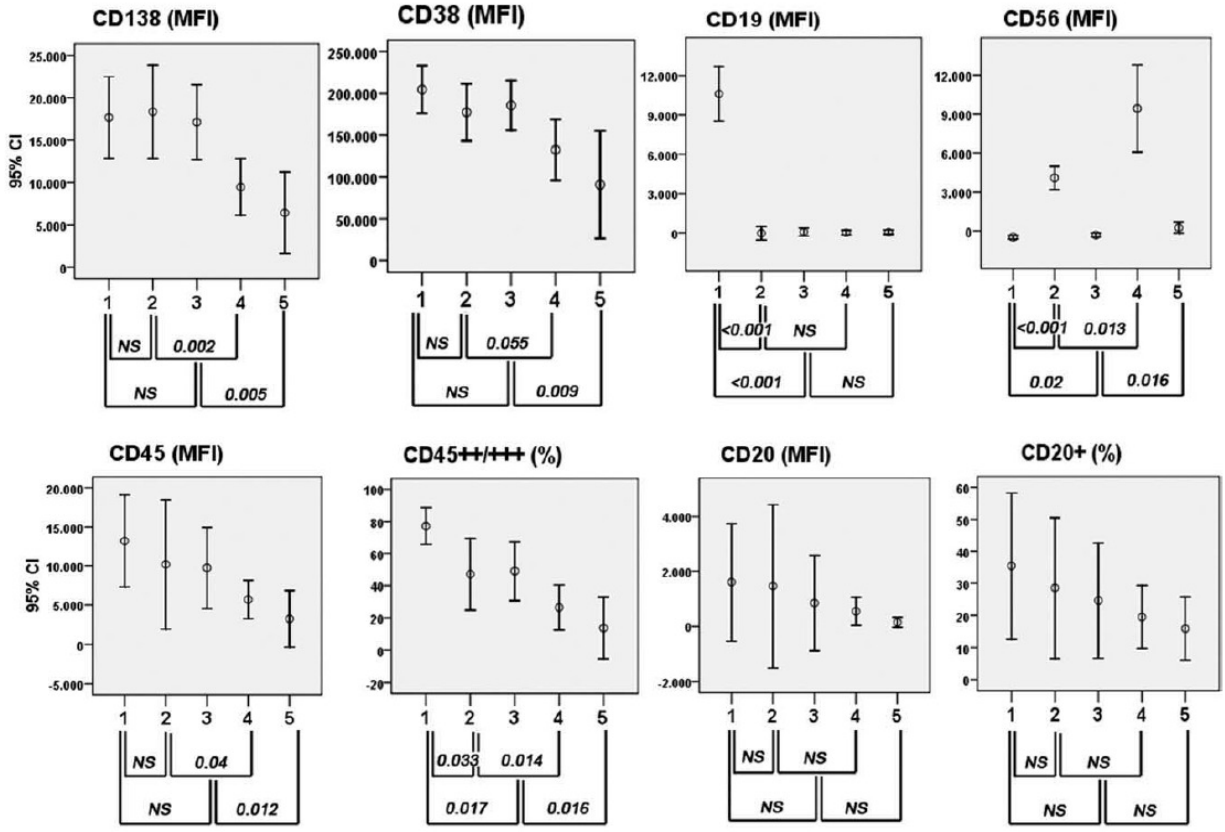
3.2. Comparission of BMPC subpopulations from healthy donors with malignant BMPC from MM patients

We further compared MFI of CD138, CD38, CD19, CD56, CD45, and CD20 as well as the proportion of PCs positive for CD20 or CD45 markers on aberrant and double-negative PCs obtained from HDs and MM patients (Fig. 3).

In our study, HDs’ aBMPCs, dnBMPCs, and nBMPCs showed similar CD138 expression. Clonal aBMPCs and dnBMPCs of MM patients had significantly lower CD138 expression compared to HDs’ respective BM PC subpopulations. It should be noted that our results contrasts the report that CD138 is overexpressed in MM.

MM PCs have been reported to express less CD38 than normal PCs. We also found that the median MFI of CD38 marker was the lowest in the malignant cell population. Similar to malignant BMPCs, aBMPC and dnBMPC of HDs had a tendency for lower CD38 expression in comparison to nBMPCs.

Fig. 3 Comparison of marker expression levels on bone marrow plasma cell subpopulations of healthy donors and myeloma patients



Mean fluorescence intensity (MFI) or mean percentage (%) with 95% confidence intervals of marker expression on bone marrow plasma cells (BMPCs): 1 - healthy donors' normal BMPCs; 2 - healthy donors' aberrant BMPCs; 3 - healthy donors' double negative BMPCs; 4 - myeloma patients' malignant aberrant BMPCs; 5 - myeloma patients' malignant double negative BMPCs.

The MFI of CD19 was similarly low in both aBMPCs and dnBMPC from HD and MM patients. Consequently, this marker cannot reliably differentiate normal BMPC expressing aberrant immunophenotype from malignant PCs.

The MFI of CD56 expression was significantly higher in clonal MM BMPC subpopulations than in respective BMPCs of HDs. Prominent hyperexpression of CD56 could serve as a valuable marker of malignancy in cases with CD19-/CD56+ malignant PC immunophenotype. In CD56 negative MM cases, malignant cells completely overlap with the normal BMPCs on the CD19/CD56 dot plot. CD56 should not be used as a marker of malignancy when exact immunophenotype of malignant clone is unknown.

The dim expression of CD45 is characteristic for malignant PCs. The MFI of CD45 and the proportion of cell with bright CD45 (++/+++) expression was lower in aBMPC and dnBMPC subpopulations compared to the nBMPC subpopulation in HDs'. In malignant BMPCs, the MFI and proportion of cells with the bright expression of CD45

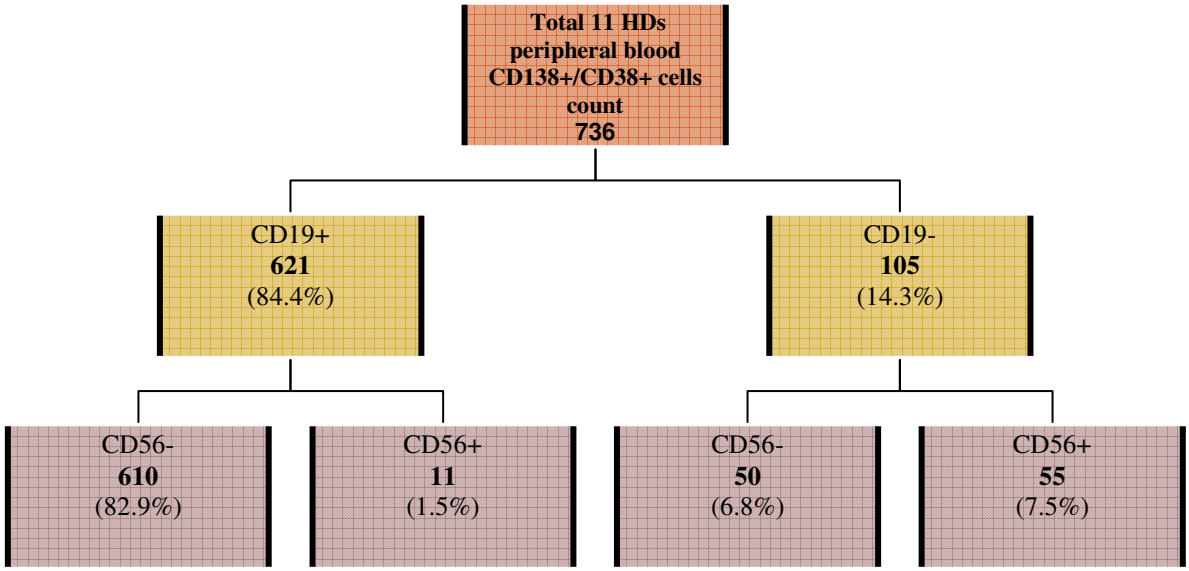
marker was even lower. A tendency for lower expression of CD45 marker on aBMPC and dnBMPC subpopulations in HDs could be misleading differentiating malignant and normal plasma cells carrying aberrant immunophenotype. We also noticed that the distribution of CD45 in the same population was heterogeneous from negative to strongly positive. In summary, we believe that CD45 marker is not helpful in differentiating normal BM PC subpopulations with CD19-/56± immunophenotype from truly malignant PCs.

The data about the expression of CD20 on PCs of healthy people is conflicting. We found that the proportion of CD20 expressing PCs was highly variable from case to case in HDs and MM patients. Neither the proportion of cells with CD20 expression nor the MFI of this marker differed significantly in the analyzed subpopulations.

3.3. Circulating plasma cells in PB of HD

We analyzed peripheral blood samples of eleven HDs for the presense of CPCs. Median analyzed TLC in the PB samples was 520940 (348810 - 1000000). We analyzed all detected CD138+/CD38+ plasma cells. 9 HDs had CPC population exceeding 20 cells and only one had more than 100 peripheral blood plasma cells. 20 cells treshold seems to be optimal with regard to assay sensitivity. Median proportion of CPCs among TLC was 0.006% (0.00 – 0.074%). Schematic representation of immunophenotypic properties of all detected CPCs presented in Fig. 4. Vast majority of detected plasma cells – 84.4% carried CD19+/CD56- immunophenotype and were classified as immunophenotypically normal (nCPC).

Fig. 4 CD19 and CD56 markers expression on HDs‘ peripheral blood plasma cells



Seven of eleven HDs has nCPC subpopulation above the predefined detection treshold of 20 cells. One of these HDs with also had 28 events carrying CD19-/CD56- immunophenotype. This subpopulation consisted only 8,4% of all CPCs‘ in the PB sample of this donor and didn‘d reached conventional 20% treshold for identification of marker expression. Moreover it looks as outcentric spread of main CD19+/CD56- CPCs subpopulation in CD19/CD56 gate and was not considered to be separate CPC

subpopulation. Hovewer this finding rised a question about insufficient specifficity of the assay using as low as 20 cells treshold. Therefore we included aditional criterion determining CPC subpopulation – it shoul consist more than 20% of all detected plasma cells. Other authors also highlighted the importance of treshold proportion from all detected PCs for the definition of malignant PC subpopulation.

CD45 marker expression was more homogenic on CPCs when compared to BMPC of HDs. Six out of seven detected nCPC subpopulations had bright (++/+++) and one HD dim (+) CD45 expression. CD45 marker could be helpfull differentiating normal and malignant CPCs.

3.4. Criteria for definition of normal and malignant CPC subpopulation

Minimal number of cells providing satisfactory intraassay variability and used as a treshold for the definition of a population in flow cytometry was defined as 20 by several investigator groups. Analyzing HDs peripheral blood plasma cells we found this treshold insufficient to warrant assay specificity. Hovewer given the rarity of CPCs in peripheral blood higher cell count defining subpopulation seems untoward. To increase specificity we defined minimal proportion of cells expressing different phenotype as aditional criterion. Expression of markers most widely used for the separation of normal an malignant plasma cells (CD19 and CD56) was found to be homogeneously normal in peripheral blood plasma cells of healthy donors. This finding, differently from BMPC, enabled to separate normal and malignant CPC using only expression of CD markers with clonality assassment being of secondary importance (Fig. 5). We defined criteria for the definition of malignant and normal circulating plasma cells based on analyses from our study and data published by other investigators.

Criteria for identification of malignant CPC subpopulation (mCPC):

1 Obligatory criteria
   a) contains ≥ 20 CD138+/CD38+ plasma cells;
   b) aberrant expression of CD19 and CD56 markers (CD19-/CD56±);
   c) aberrant subpopulation constitute ≥ 20% of total detected CD138+/CD38+ plasma cells;

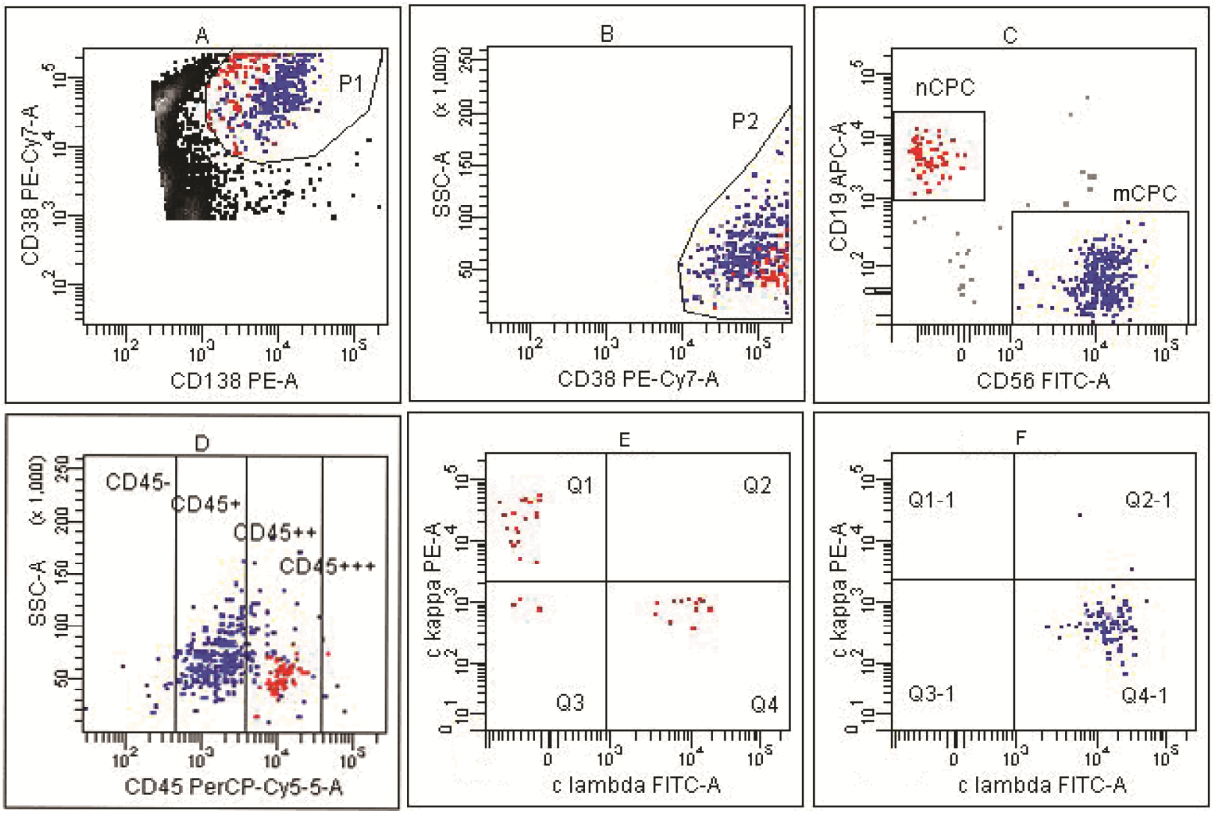
2 Secondary criteria
   a) clonal expression of cytoplasmic light chains, kappa/lambda <1/3 or >4/1.

Criteria for identification of normal CPC subpopulation (nCPC):

1 Obligatory criteria
   a) contains ≥ 20 CD138+/CD38+ plasma cells;
   b) CD19+/CD56- immunophenotype;

2 Secondary criteria
   a) polyclonal expression of intracytoplasmic light chains, kappa/lambda >1/3 and <4/1;
   b) bright expression of CD45 marker

Fig. 5 6-colour flow cytometric analysis of peripheral blood circulating plasma cell subsets

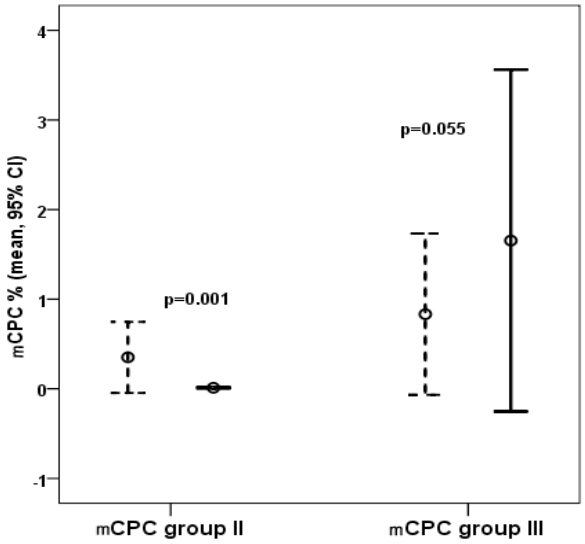


Peripheral blood circulating plasma cells (CPC) were identified by gating on CD38 vs. CD138 (A) and backgating on side scater (SSC) vs. CD38 (B). Acquired CD138+/CD38+ plasma cells were then tested for the expression of CD19, CD56 and CD45 markers, thus defining "normal" (nCPC, in red) and "aberrant" (mCPC, in blue) subpopulations (C and D). The cytoplasmic light chain expression of CPCs was tested to confirm polyclonality (E) of nCPCs or monoclonality (F) of mCPCs

3.5. Quantitative characteristics of mCPC in RR MM patients and it's kinetics in response to one chemotherapy cycle

mCPC subpopulation based on aforementioned criteria were detected in 24 (57.1%) patients before treatment (Fig. 5). Median mCPC proportion in cases with detectable subpopulation was 0.086% (0.003 – 4.1). mCPC were detected in 22 cases (52.4%) after one treatment cycle. Based on predefined criteria patients were allocated into one of three groups: patients with no detectable mCPCs in both pre and postchemotherapy samples (group I, N 15), patients with a decrease in mCPCs postchemotherapy as compared to mCPCs before chemotherapy (group II, N 14) and patients with no change or increase in mCPCs postchemotherapy as compared to mCPCs before chemotherapy (group III, N 13) (Table 1). mCPC kinetics in the groups II and III are depicted in Fig. 6.

Fig. 6 mCPC kinetics in patients from groups II and III before and after the first therapy cycle



Dotted line - mCPC before chemotherapy, solid line mCPC after first chemotherapy cycle

Table 1 Patient characteristics

		All patients at baseline	mCPC Kinetic Group			
			I	II	III	P*
Number of patients, N		42	15	14	13	
Age, median (range)		57 (39-74)	56(39-72)	62 (49-73)	56 (45-74)	0.23
Treatment lines, median (range)		2 (1-5)	2 (1-4)	2 (1-4)	2 (1-5)	0.53
Previous HDT, N		16	5	4	7	0.37
Months since diagnosis, median (range)		25 (3-172)	26 (6-172)	21 (3-147)	37 (3-84)	0.56
Beta 2 microglobulin, mmol/l, median (range)		4.01 (2.0 – 45.4)	2.49 (2.0-9.96)	3.86 (2.2-45.4)	4.9 (2.3-12.9)	0.07
Albumin g/l, median (range)		43.3 (24.4 – 53.0)	44.7 (36.1-50.1)	40.0 (32.1-53.0)	43.1 (24.4-50.9)	0.13
Hemoglobin g/l, median (range)		113 (72-153)	117 (96-153)	110 (76-141)	121 (72-140)	0.34
Platelets 10^9/l, median (range)		219 (12-408)	250 (108-344)	173 (12-408)	157 (19-322)	0.1
BM plasma cells, %, 10^9/l, median (range)		13.5 (0.0-89)	12.5 (0.5-48)	10.3 (0-65)	27.5 (0.5-89)	0.15
Creatinine, mcmol/l, median (range)		86.5 (44-540)	84 (52-292)	93.5 (44-540)	87 (64-267)	0.68
Ca^{++}, mmol/l, median (range)		1.25 (1.1-1.95)	1.21 (1.12-1.3)	1.26 (1.21-1.58)	1.25 (1.1-1.95)	0.12
Chemotherapy	B DB PAD VAD		12 1 1 1	10 1 1 2	13 0 0 0	0.62
EBMT response achieved after I-st treatment course	PD SD MR PR		0 5 4 5	0 3 4 6	3 4 4 1	0.11
Early progression			1(6.7%)	0 (0%)	11 (84.6%)	<0.001

- p value for comparison among mCPC kinetics groups

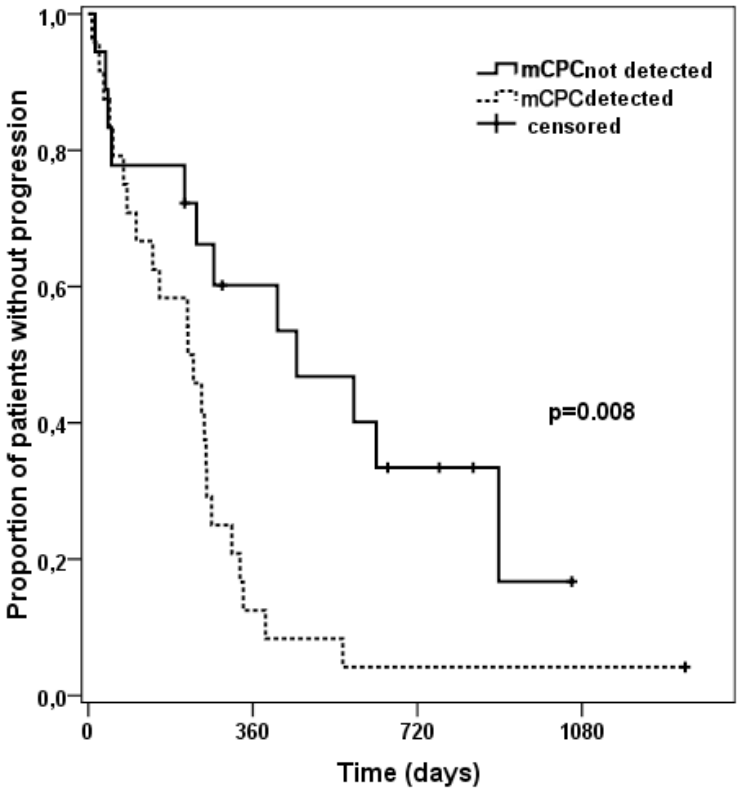
Group I - patients with no detectable mCPCs in both pre and postchemotherapy samples, group II - patients with a decrease in mCPCs postchemotherapy as compared to mCPCs before chemotherapy, group III - patients with no change or increase in aCPCs postchemotherapy as

compared to mCPCs before chemotherapy, mCPC – malignant circulating plasma cells, VAD – vincristin, doxorubicin, dexamethasone, PD – progressive disease, SD - stable disease, MR – minimal response, PR partial response

3.6. Prognostic value of pretreatment mCPC detection

We evaluated the prognostic value of mCPCs detection before treatment. Patients with detectable pretreatment mCPCs had significantly inferior median TTP of 218 vs. 456 days compared to those with the absence of detectable preatreatment mCPCs (p=0.008), but not OS (p=0.16). (Fig. 7)

Fig.7 Time to progression in patients with detectable and undetectable mCPCs before treatment.

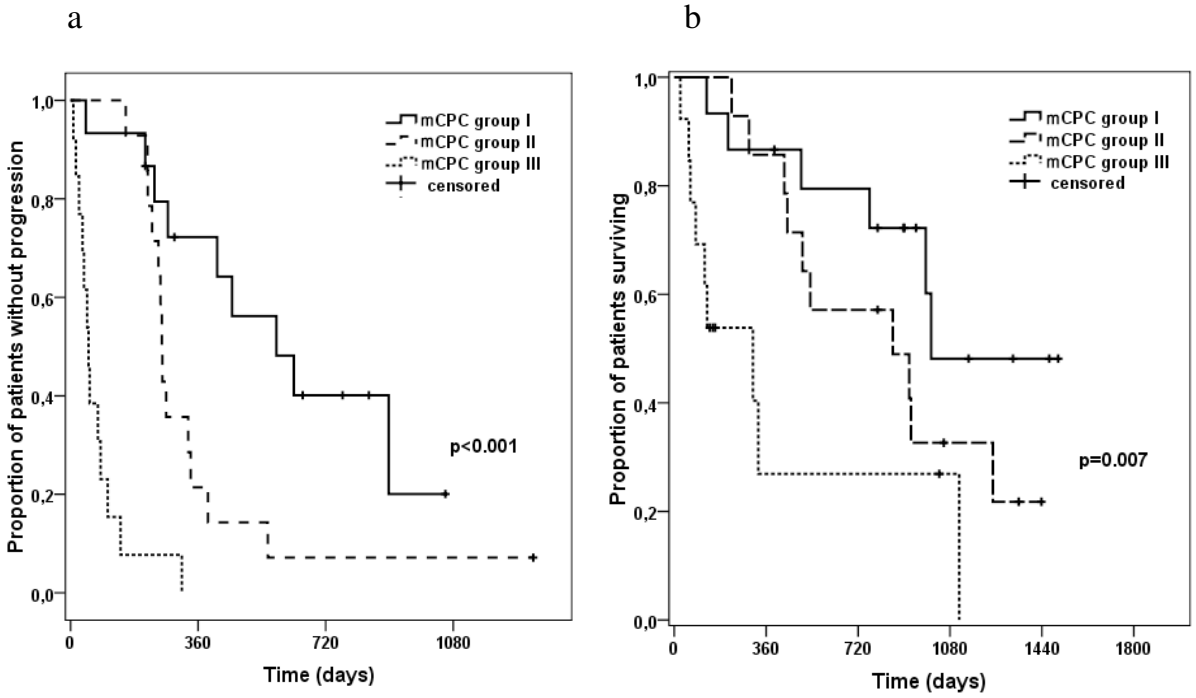


3.7. Prognostic value of mCPC kinetics pattern for EP, TTP and OS

We tested the hypothesis that failure to achieve mCPC reduction after one treatment course could identify patients who are refractory to treatment and are at risk for early progression (EP). 11 (84.6%) patients from mCPC kinetics group III and only one (3.4%) patient outside of mCPC group III had EP (Table 1). ROC analysis reveald that nonreduction of mCPC after one treatment cycle predicted early progression with a sensitivity of 91.7% and specificity of 93.3%. ROC AUC of the assay was 0.91 ($p<0.001$). In multivariate analysis (logistic regression), stepwise procedure selected the only significant factor for EP – mCPC kinetic groups (p=0.003). Odds ratio for EP in mCPC group III patients comparing to mCPC group I was 77 (95% CI 6.2-963.7, $p<0.001$). Using logistic regression analysis of patients with detectable mCPCs we found that mCPC2/mCPC1 ratio of 0.807 could predict EP with the highest sensitivity and the specificity (100% and 93.8%, respectively).

We assessed the three mCPC kinetic groups in relation to TTP and OS. TTP differed significantly with the medians of 581, 258 and 51 days in groups I, II and III, respectively ($p<0.001$) (Fig 8a). Pairwise comparison of TTP using Kaplan-Meier method showed that differences between groups I and II, II and III, I and III all were significant ($p=0.02$, $p<0.001$ and $p<0.001$, respectively). Since the consolidation with high-dose chemotherapy and autologous stem cell transplantation before disease progression could have introduced a bias in favor of the transplanted patients, we censored thirteen patients at the time of transplantation but the difference in TTP remained significant for all three groups ($p<0.001$ in overall comparison).

Fig. 8 Time to progression (a) and overall survival (b) in three aCPC kinetic groups



We found that OS was significantly different between the groups I vs III and II vs III (median OS 1006 vs. 308 days and 856 vs. 308 days respectively ($p=0.009$ and 0.036) (Fig. 8b). In univariate analysis mCPC group, creatinine concentration, beta 2 microglobulin were significantly associated both with TTP and OS. BM PC % was associated only with TTP, while the presence of extramedullary disease and hemoglobin level were assotiated only with OS. Multivariate analysis reveald that mCPC group and baseline creatinine concentration were independent factors significantly influencing both TTP and OS (Table 2).

Table 2 Multivariate analysis of prognostic factors for TTP and OS

	Time to progression		Overall survival	
	HR (95% CI)	p value	HR (95% CI)	p value
mCPC group		<0.001		0.005
I	Reference			
II	2.2 (0.8-5.8)	0.12	1.6 (0.51-5.0)	0.43
III	17.4 (5.8-52.4)	<0.01	5.8 (1.8-17.9)	0.003
Creatinine	1.005 (1.001 - 1.008)	0.019	1.006 (1.002 - 1.01)	0.002

HR – hazard ratio, CI – confidence interval

3.8. Comparisson of standart response criteria and mCPC kinetics pattern

We assessed the EBMT response after one treatment cycle and then after three and six months from the initiation of treatment. EBMT response category after the first treatment cycle was not predictive for EP. 5 of 12 (41.7%) patients who progressed early, achieved ≥ minimal EBMT response after the first chemotherapy cycle (Table 1). Early EBMT response defined as at least a minimal EBMT response evaluated before the second treatment cycle was not predictive of either TTP or OS (p=0.32 and p=0.65, respectively). Grouping according to mCPC kinetics correlated significantly with EBMT response at 3, 6 months and maximal response (r values - -0.51, -0.64, -0.51 and p values - 0.001, <0.001, 0.001 respectively), but not with the EBMT response after the first treatment cycle (r= -0.28, p=0.09). Maximal responses defined by EBMT criteria were almost identical in aCPC kinetics groups I and II since 80.0% and 86.7% of patients from groups I and II respectively achieved partial remission (PR) or better. Among group III patients with non-decreasing mCPCs, only 1 patient achieved PR.

3.9. Quantitative characteristics of normal PC subpopulation in PB and BM of RR MM patients

Data about nPC population were available from 40 RR MM patients. We were able to confirm the presence of plasma cells in bone marrow of patients by morphology under the light microscope (LM) in all but one patient with predominant extramedulary disease. Median proportion of plasma cells in BM by LM was 12.5% (0 - 89%).

Multiparameter flow cytometry revealed plasma cells in bone marrow samples of all 40 patients. Majority of these cells were malignant and expressed aberrant phenotype. Median proportion of such cells in the sample was 8.0% (0.05 - 87.9). 32 MM patients (80%) had detectable nBMPC (CD19+/CD56-) subpopulation. Median proportion of detected nBMPC subpopulation was 0.04% (0–1.51) of TLC in the sample. Mediana BMPC/nBMPC ratio was 225 in study patients. nBMPC proportion in MM patients was decreased as compared to HC (Table 3).

Peripheral blood circulating plasma cells were detected in 33 cases out of 40 (82.5%) before treatment. In 23 (57.5%) cases we were able to identify immunophenotypically normal CPC. mCPC subpopulation were detected in 24 MM patients. Proportion of both CPC subpopulations was very low, however in patients with detectable CPC, nCPC were observed in approximately 1 log smaller proportions, than mCPC: mean 0.025±0.034% and mean of 0.72±1.2%. nCPC subpopulation were

identifiable in similar proportion of HD as in MM patients – 7 of 11 cases (63.6%). Size of detected nCPC subpopulations in MM patients and HC were also similar (Table 3).

Table 3 Proportion of normal plasma cell subpopulation in peripheral blood and bone marrow of MM multiple myeloma patients and healthy donors

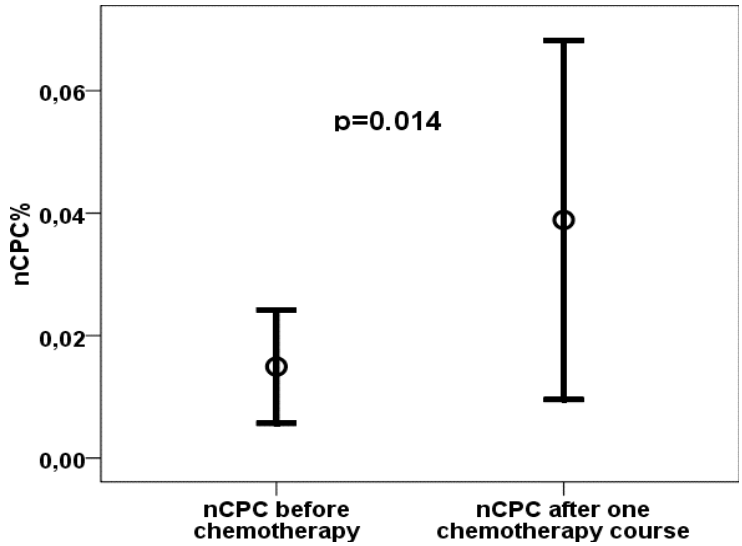
		nCPC CD19+/CD56-	**nBMPC CD19+/CD56-**
MM patients N 40	Mean	0.015%	0.11%
	Median	0.006%	0.04%
Mann – Whitney Test		p=0.48	p=0.001
Healthy donors N 11	Mean	0.01%	0.21%
	Median	0.003%	0.20%

nCPC – immunophenotypically nor mal plasma cells nBMPC – immunophenotypically normal bone mar row plasma cells

3.10. Clinical value of nCPC and nBMPC detection in RR MM patients

nCPC population, whether detected or not at the begining of treatment had no significant assotiations neither with disease burden (defined as bone marrow plasma cells by microscopy or FC) nor biological properties (reflected by ß2 microglobulin, albumin, hemoglobin levels and renal function). Detection of baseline nCPC was also not prognostic for TTP and OS. We were able to identify only weak inverse correlation of nCPC proportion with MM patients age (r=-0.34, p=0.03). We also looked at kinetics of MM patients' nCPC in response to one treatment course. We found proportion of nCPC significantly lower at baseline than after one treatment course – median 0.006% vs 0.013% respectively (p=0.014) (Fig. 9). Patients with increased nCPC had prolonged TTP in comparison to patients with decreased or absent nCPC: median of 339 vs 105 days (p=0.038) respectively. OS was not different in both groups.

Fig. 9 Kinetics of immunophenotypically normal circulating plasma cells (nCPC) in response to one chemotherapy course

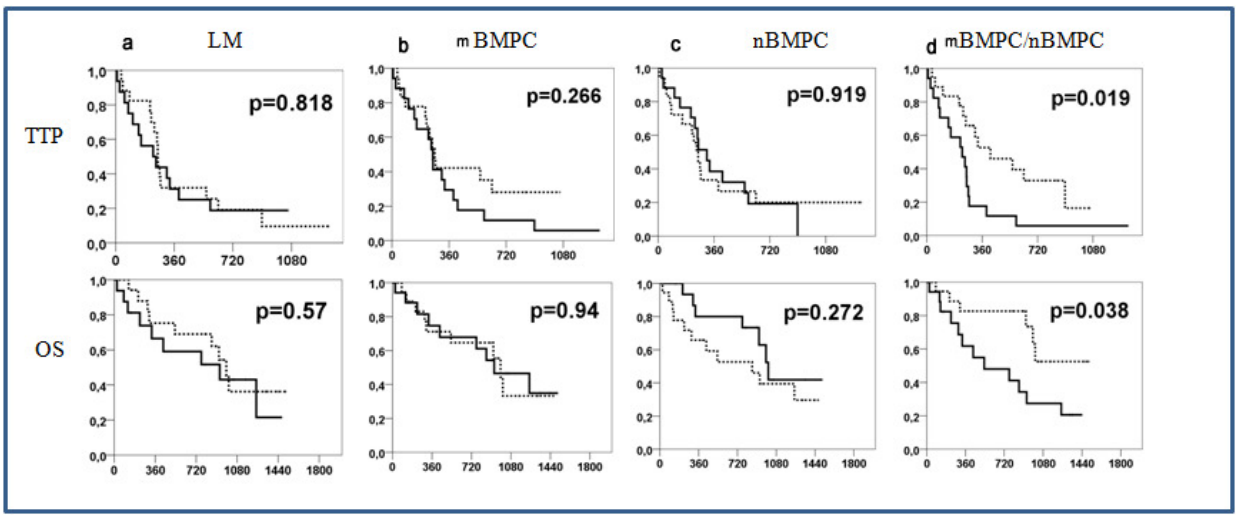


Error bars represents 95% confidence interval for mean.

We evaluated prognostic impact of residual bone marrow nCPC in refractory or relapsed MM patients. Five patients with predominantly extramedullar relapse and low BM involvement were excluded from this analysis, as BM analysis in these cases was considered to be not informative in respect to disease activity. To test prognostic value of mBMPC/nBMPC ratio in univariate fashion we dichotomised patients in two groups according to the median value of mBMPC/nBMPC ratio, which was found to be 225. TTP and OS both were significantly longer in patients with higher nBMPC proportion median 414 vs 230 days (p=0.019) and median not reached vs days (p=0.038), respectively (Fig. 10 d). Markers related to disease activity and prognosis have more favourable profile in patients with higher nBMPC proportion: lower ß2 microglobulin (p=0.006), BMPC by LM (p=0.019) and calcium (p=0.045), higher hemoglobin (p=0.03) and platelets (0.002). Further we compared prognostic impact on TTP and OS of pretreatment BM samples analysis by two different methods - LM and MFC. We dichotomize study patients into two groups using median values of following variables: BMPC detected by LM and three variables established by MFC - nBMPC, mBMPC proportion of TLC and mBMPC/nBMPC ratio. mBMPC/nBMPC ratio was the only variable significantly assotiated with TTP and OS. Grouping according to median value of BMPC detected by LM was not prognostic neither for TTP nor OS (Fig. 10).

We built Cox regression model using mBMPC/nBMPC ratio as continuous variable for multivariate analysis. Such established MM risk factors as age, ß2 microglobulin, creatinine, time since diagnosis, previous treatment lines, hemoglobin concentration were also evaluated in this model. mBMPC/nBMPC ratio was found to be significant and independant of aforementioned risk factors, predictor for TTP (p=0.014) and OS (p=0.003).

Fig 10 Prognostic impact of bone marrow plasma cell content analysis by different methods



LM – light microscopy, mBMPC – malignant bone marrow plasma cells, nBMPC – normal bone marrow plasma cells, TTP –time to progression, OS – overall survival

4. CONCLUSIONS

1. Aberrant by CD19 and CD56 markers expression (CD19-/56+ and/or CD19-/CD56-), but polyclonal by light chain expression bone marrow plasma cell subpopulations containing more than 100 cells, are detectable in bone marrow of healthy donors, using 6-colour FC methodology.

2. Expression of CD19, CD56, CD45, CD20, CD38 markers, could not reliably differentiate between polyclonal healthy donors and MM patients malignant CD19-/CD56+ and CD19-/CD56- bone marrow plasma cell subpopulations. In cases with low aberrant plasma cell burden it is recomended to analyse intracytoplsmic light chain expression for clonality assessment.

3. Aberrant by CD19 and CD56 markers expression plasma cell subpopulations containing ≥20 cells and consisting ≥ 20% of all detected in sample plasma cells, were undetectable in peripheral blood of healthy donors.

4. Detection of malignant CPC in pretreatment samples of RR MM patients is of negative prognostic value. Patietns with detectable mCPC before treatment had significantly shorter TTP as compared to patients with undetectable mCPC (p=0.008).

5. Failure to achieve mCPC reduction after the first treatment cycle is assotiated with refractoriness to given treatment and confer poor prognosis in RR MM patients. Esatblishment of mCPC kinetics after first therapy cycle could identify patients refractory to given therapy who are at risk for early progression more precisely than a standart response criteria (EBMT).

6. Increase of nCPC proportion after first chemotherapy cycle is assotiated with prolonged TTP as compared to patients with not changed or decreased nCPC subpopulation (p=0,038).

7. Pretreatment BMPC analysis using MFC was shown to be of higher prognostic value than PC quantification employing light microscopy. mBMPC/nBMPC ratio established by MFC was identified as significant and independant prognostic marker for TTP and OS while estimation of BMPC proportion by LM was not significantly assotiated neither with TTP nor OS.

5. PUBLICATIONS

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3. Peceliunas V, Janiulioniene A, Matuzeviciene R, Griskevicius L. Circulating plasma cells (CPCs) predict the outcome of relapsed or refractory multiple myeloma (RR MM) [abstract]. Haematologica 2010;95(Suppl 2):396, abs. 0954.

4. Peceliunas V, Matuzeviciene RK, Janeliuniene M, Griskevicius L, Kucinskiene ZA. Evaluation of Circulating Plasma Cell Count Kinetics in the Treatment of Relapsed/Refractory Myeloma. Cytometry Part B: Clinical Cytometry 2008; 74B(6): 409-10, abs. P-10-118.

5. Peceliunas V, Janiulioniene A, Matuzeviciene R, Zvirblis T, Griskevicius L. Circulating plasma cells predict the outcome of relapsed or refractory multiple myeloma. Leukemia & Lymphoma. [Submitted for publication].

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7. SUMMARY IN LITHUANIAN

PLAZMINIŲ LĄSTELIŲ IMUNOFENOTIPINĖS ANALIZĖS REIKŠMĖ VERTINANT MIELOMINĖS LIGOS RIZIKĄ IR ATSAKĄ Į GYDYMĄ

Įvadas

Didžiulė pastarojo meto pažanga ML gydymo srityje, vaistų turinčių naują veikimo mechanizmą įdiegimas stipriai paveikė ML prognozę. Tai privertė mokslininkus iš naujo įvertinti ML stadizaciją, rizikos faktorius. Pastaruoju metu ML rizikos vertinimui imta taikyti ir tėkmės citometrijos (TC) metodą. Neseniai publikuotame ekspertų bendro sutarimo dokumente apibrėžta TC naudojimo ML rizikos vertinimui sritys. ML rizikos nustatymui rekomenduota vertinti normalių ir piktybinių plazminių ląstelių santykį kaulų čiulpuose, imunofenotipinių žymenų raišką (CD117, CD28 ir kt.). Cirkuliuojančių plazminių ląstelių (CPL) prognostinė reikšmė įvardinta kaip perspektyvi klinikinių tyrimų sritis. Iki šiol nėra pateikta duomenų apie CPL nustatymo prognostinę reikšmę recidyvavusiai mielominei ligai. Standartiniai ML atsako į gydymą vertinimo metodai daugiausiai remiasi surogatinio ligos masės žymens - paraproteino kinetika. Dėl ilgo skilimo pusperiodžio paraproteino kinetika pasižymi inercija ir atsakas vertinamas ne anksčiau kaip po 2 – 3 mėn. nuo gydymo pradžios. Mes iškėlėme hipotezę, kad pCPL galėtų būti naudojamos kaip tiesioginis ligos masės ir aktyvumo žymuo vertinant atsaką į gydymą. Kadangi pCPL yra piktybinio ML klono dalis, jų kinetika neturėtų paraproteinui būdingos inercijos. Kadangi normalių ir piktybinių plazminių ląstelių proporcijos kaulų čiulpuose daro priešingą įtaką prognozei, atliekant tyrimą reikėjo optimizuoti TC metodologiją skiriant normalias ir piktybines plazmines ląsteles. Dėl šios priežasties tyrimui naudojome naują iki tol neaprašytą imunofenotipinių žymenų derinį apimantį svarbiausius plazminių ląstelių identifikavimo (CD138, CD38, CD45), nenormalios (aberantinės) raiškos (CD19, CD20 ir CD56) ir kloniškumo žymenis (kappa ir lambda intracitoplazminės lengvosios grandinės). Tirdami sveikus žmones siekėme patikslinti normalių PL imunofenotipines sąvybes bei aprašyti normalių ir piktybinių periferinio kraujo PL atskyrimo metodiką.

Darbo tikslas

Įvertinti plazminių lastelių imunofenotipavimo tėkmės citometrijos metodu reikšmę, nustatant pažengusios mielominės ligos ankstyvą atsaką į gydymą ir prognozę.

Darbo uždaviniai

1. Įvertinti sveikų donorų periferinio kraujo ir kaulų čiulpų plazminių ląstelių sinchroninę CD19, CD56 ir CD45 žymenų raišką.
2. Ištirti sveikų donorų plazminių ląstelių subpopuliacijų, pasižyminčių atipine CD19 ir CD56 žymenų raiška, kiekybines ir imunofenotipines savybes bei palyginti jas su mielomine liga sergančių pacientų piktybinių plazminių ląstelių subpopuliacijomis.

3. Nustatyti periferiniame kraujyje cirkuliuojančių normalių ir piktybinių plazminių ląstelių subpopuliacijų imunofenotipinius kriterijus.
4. Įvertinti piktybinių cirkuliuojančių plazminių ląstelių nustatymo prieš gydymą prognostinę reikšmę pacientams, sergantiems pažengusia mielomine liga.
5. Išanalizuoti piktybinių cirkuliuojančių plazminių ląstelių kinetiką po pirmojo chemoterapijos kurso, jos reikšmę, nustatant atsparumą skiriamam gydymui bei prognozuojant ligos išeitis.
6. Ištirti mielomine liga sergančių pacientų periferiniame kraujyje cirkuliuojančių normaliu plazminių ląstelių kiekybines charakteristikas ir prognostinę reikšmę reikšmę
7. Nustatyti normalių ir piktybinių kaulų čiulpų plazminių ląstelių santykio vertę prognozuojant pažengusios mielominės ligos išeiti

Darbo naujumas

Optimizavome tėkmės citometrijos metodiką atskiriant normalias ir piktybines plazmines ląsteles, pritaikę iki tol nenaudotą imunofenotipinių žymenų derinį. Ištyrėme ir aprašėme nevienareikšmiškai vertintą normalių plazminių ląstelių CD19, CD56 ir CD45 žymenų raišką. Sukūrėme iki tol praktikoje nenaudotą ankstyvo atsako į gydymą vertinimo metodą, įgalinantį anksčiau nei standartiniais metodais nustatyti pacientus, kurių ML yra atspari skiriamam gydymui. Mūsų duomenis validavus didesnėse studijose šis metodas galėtų tapti klinikiniu standartu gydant pažengusią mielominę ligą. Nustatėme, kad pCPL aptikimas prieš gydymą yra nepalankios prognozės žymuo pažengusia mielomine liga sergantiems pacientams. Šis žymuo atlikus papildomus tyrimus galėtų būti naudojamas gydymo taktikos nustatymui. Mes pirmieji aprašėme ML pacientų periferinio kraujo normalių plazminių ląstelių subpopuliacijos kiekybines charakteristikas ir kinetiką gydymo metu. Taip pat įvertinome KČ PL imunotipavimo prognostinę reikšmę bei palyginome ją su KČ aspirato mikroskopavimo reikšme.

Metodai ir tiriamieji

Į tyrimą įtraukėme suaugusius pacientus sirgusius atsparia gydymui ar recidyvavusia mielomine liga. Buvo įtraukti 42 pacientai. Į tyrimą įtraukėme 11 sveikų donorų kaip kontrolinę grupę, tėkmės citometrijos metodologijos optimizavimui.

Periferinio kraujo ir kaulų čiulpų plazminių ląstelių imunofenotipavimui taikėme 6 spalvų tėkmės citometrą – FACSCanto. Naudojome originalią iki tol neaprašytą TC metodiką, visi mėginiai buvo dažomi 2 žymenų deriniais: CD56 / CD138 / CD45 / CD19 / CD38 / CD20 ir cLambda / cKappa / CD138 / CD38 / CD56 / CD19.

Rezultatai

Siekdami optimizuoti normalių ir piktybinių PL atskyrimą ištyrėme 11 sveikų donorų kaulų čiulpų ir periferinio kraujo mėginius. Įvertinome sinchroninę CD19/CD56 žymenų raišką plazminių ląstelių subpopuliacijose identifikuotose pagal šoninę, tiesinę

šviesos sklaidą bei imunofenotipinių žymenų CD138/CD38/CD45 raišką. Nustatėme, kad visų sveikų donorų kaulų čiulpuose aptinkamos netipinę žymenų raišką (CD19-/CD56+ ir/ar CD19-/CD56-) turinčios plazminių ląstelių subpopuliacijos.Šios subpopuliacijos sudarė apie 1/3 visų sveikų donorų kaulų čiulpuose aptiktų plazminių ląstelių. Palyginome sveikų donorų atipinių plazminių ląstelių subpopuliacijų ir mielomine liga sergančių pacientų piktybinių plazminių ląstelių imunofenotipines savybes. CD19, CD56, CD45, CD20, CD38 žymenų raiška neleido patikimai atskirti normalių atipinę žymenų raišką turinčių ir piktybinių plazminių ląstelių. CD138 žymens raiška mūsų tyrime buvo reikšmingai didesnė ant normalių atipinių plazminių ląstelių nei ant piktybinių plazmocitų, šie mūsų rezultatai nesutapo su anksčiau publikuotais duomenimis. Įvertinus lengvųjų kappa ir lambda grandžių raišką nustatėme, kad visos normalių plazminių ląstelių subpopuliacijos pasižymėjo poliklonine ekspresija, kai tuo tarpu piktybinės plazminių ląstelių subpopuliacijos ekspresavo tik kappa ar lambda lengvasias grandis. Patikimam kaulų čiulpų normalių ir piktybinių plazminių ląstelių atskyrimui, kai daugiausia remiamasi CD19 ir CD56 žymenų ekspresijos skirtumais reikalingas ir lengvųjų grandžių ekspresijos nustatymas.

Ištyrę sveikų donorų periferinio kraujo mėginius taikydami tą pačią tėkmės citometrijos metodiką, radome, kad plazminių ląstelių CD19 ir CD56 žymenų raiška yra žymiai homogeniškesnė. Mes nenustatėme atipiškai šiuos žymenis ekspresuojančių plazminių ląstelių subpopuliacijų turinčių ≥ 20 ląstelių ir sudarančių bent 20% visų mėginyje aptiktų plazmocitų. Sveikų donorų periferinio kraujo plazminių ląstelių subpopuliacijos turėjo polikloninę lengvųjų grandžių ekspresiją. Gautus duomenis toliau naudojome analizuodami mielomine liga sergančių pacientų periferinio kraujo plazminių ląstelių subpopuliacijas.

Mielomine liga sergančių pacientų periferinio kraujo mėginiuose piktybinės cirkuliuojančių plazminių ląstelių (pCPL) subpopuliacijos aptiktos pas 24 iš 42 (57,1%) ML sergančius pacientus. Pacientu, kuriems prieš gydyma buvo aptiktos pCPL medianinis LIP buvo 218 dienu, kai tuo tarpu pacientu, kuriems pCPL neaptikta medianinis LIP buvo 456 dienos (p=0,008).
Įvertinome pCPL kinetikos pirmojo chemoterapijos kurso metu klinikinę reikšmę. Pacientai pagal pCPL kinetikos pobūdį taikant iš anksto apibrėžtus kriterijus suskirstyti į tris grupes: pacientai, kurių mėginiuose pCPL neaptiktos nei prieš gydyma, nei po gydymo (I grupė, N15), pacientai, kuriems po pirmojo chemoterapijos kurso pCPL kiekis sumažėjo bent 50% (II grupė, N14) ir pacientai, kurių pCPL nesumažėjo ar padidėjo (III grupė, N13). Nustatėme, kad pCPL proporcijos nesumažėjimas ar padidėjimas po pirmojo chemoterapijos kurso, patikimai identifikuoja gydymui atsparius atvejus ir su 91,7% jautrumu bei 93,3% specifiškumu prognozuoja ankstyvą progresiją. Pacientai, kurių pCPL gydymo metu nemažėjo turėjo statistiškai reikšmingai trumpesnį LIP ir bendrą išgyvenamumą (BI), nei pacientai, kurių pCPL sumažėjo ar buvo neaptinkamos (medianinis LIP buvo atitinkamai 581, 258 ir 51 diena, p<0.001, medianinis BI - 1006, 856 ir 308 dienos, p=0,007). Ankstyvas (po pirmojo chemoterapijos kurso) atsako į gydymą vertinimas taikant standartinius kriterijus (EBMT) nebuvo prognostiškai informatyvus. Daugiamatė analizė patvirtino, kad pCPL kinetikos pobūdžio nustatymas buvo statistiškai reikšmingas nepriklausomas prognostinis LIP, BI ir ankstyvos progresijos žymuo.

Tyrėme mielomine liga sergančių pacientų normalių plazminių ląstelių subpopuliacijų nustatymo klinikinę reikšmę. Nustatėme, kad ML pacientų normalių CPL

(nCPL) kiekybinės charakteristikos nesiskyrė nuo sveikų donorų. nCPL aptikimas ML pacientams prieš gydymą nebuvo prognostiškai reikšmingas. Tačiau nCPL kiekio padidėjimas po pirmojo chemoterapijos kurso buvo susijęs su pailgėjusiu laiku iki progresijos.
CD19+/CD56- poliklonin kaulų čiulpų plazminių ląstelių subpopuliacija (nKČPL) buvo aptinkama visų ML pacientų kaulų čiulpų mėginiuose. Ligos aktyvumo ir rizikos veiksniu charakteristikos buvo palankesnės pacientų su didesne nKČPL proporcija grupėje: mažesnė beta2 mikroglobulino koncentracija (p=0,006), mažesnė KČPL infiltracija mikroskopuojant (p=0,019), žemesnė jonizuoto kalcio koncentracija (p=0,045), didesnis hemoglobino kiekis (p=0,03), daugiau trombocitų (p=0,002). Nustatėme, kad tėkmės citometrijos metodu nustatytas nKČPL ir piktybinių plazminių ląstelių santykis kaulų čiulpuose buvo prognostiškai reikšmingas vertinant LIP ir BI, kai tuo tarpu šviesos mikroskopijos būdu nustatyta kaulų čiulpų plazminių ląstelių infiltracija nebuvo prognostiškai reikšminga.

Išvados

1. Sveikų donorų kaulų čiulpuose aptinkamos >100 ląstelių turinčios ir aberantine CD19 bei CD56 žymenų ekspresija pasižyminčios poliklonins plazminių ląstelių populiacijos: CD19-/56+ ir/ar CD19-/CD56-.

2. CD19, CD56, CD45, CD20, CD38 žymenų ekspresijos skirtumai ant sveikų žmonių poliklonini bei mielomine liga sergančių žmonių piktybinių CD19-/56+ ir CD19-/CD56- plazminių ląstelių subpopuliacijų yra nepakankami, kad užtikrintu šių populiacijų atskyrimą. Tais atvejais, kai atipinė plazmocitų populiacija yra maža, būtinas lengvųjų grandžių ekspresijos vertinimas monokloniškumui patvirtinti.

3. Kontrolinės sveikų asmenų grupės periferinio kraujo mėginiuose nenustatėme aberantine CD19 ir CD56 žymenų raiška pasižyminčių plazminių ląstelių subpopuliacijų, kurios turėtų daugiau nei 20 ląstelių ir/ar sudarytų > 20% visų aptiktų CD138+/CD38+ plazminių ląstelių.

4. Piktybinių CPL aptikimas prieš gydymą yra nepalankus prognostinis žymuo pacientams sergantiems atsparia gydymui ar recidyvavusia mielomine liga. pCPL aptikimas prieš gydymą šiems pacientams buvo susijęs su statistiškai reikšmingai trumpesniu LIP (p=0,008).

5. pCPL proporcijos nesumažėjimas po I-ojo chemoterapijos kurso yra susijęs su ligos atsparumu skiriamam gydymui, ir yra nepriklausomas labai blogos prognozės žymuo. pCPL kinetika geriau nei standartiniai atsako vertinimo kriterijai po pirmojo chemoterapijos kurso identifikuoja pacientus atsparius gydymui ir prognozuoja ankstyvą progresiją.

6. nCPL proporcijos didėjimas po pirmojo gydymo kurso susijęs su palankia prognoze. Pacientų, kurių nCPL proporcija didėjo, LIP buvo statistiškai reikšmingai ilgesnis nei pacientų su sumažėjusia ar neaptinkama nCPL subpopuliacija (p=0,038).

7. Kaulų čiulpų mėginio imunofenotipavimas pažengusia mielomine liga sergantiems pacientams yra prognostiškai informatyvesnis nei mikroskopavimas. Nustatėme, jog

didėjantis piktybinių KČPL/ nKČPL santykis yra statistiškai reikšmingas, nepriklausomas sutrumpėjusio LIP bei BI žymuo, tačiau mikroskopuojant nustatyta kaulų čiulpų plazminių ląstelių proporcija nebuvo prognostiškai reikšminga.