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Original paper

The morphology of myeloma cells changes with progression of the disease

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Aim of the study: Multiple myeloma is a heterogeneous entity with variable course. Plasma cells found in bone marrow smears are characterised by extremely high diversity of morphology. We have attempted to determine whether the morphological characteristics of myeloma cells vary with the natural course of the disease. We investigated the incidence of selected morphological features and planimetric parameters of myeloma cells present in bone marrow smears.

Material and methods: Material collected from 103 patients was evaluated at diagnosis and then during relapse. It was found that in the same patients, plasma cell morphology changes in the course of the disease: cell surface, nucleus surface, tumour cell anisocytosis and nuclear-cytoplasmic ratio increase significantly.

Results: The results suggest that some morphological features are more common in clinically advanced disease. These include the number of nucleoli, the number of myeloma cells with irregular nuclei, and larger nuclei. Using the classification systems according to Greipp and Goasguen, we have noted changes in morphological pattern of myeloma cells in some patients with progressive multiple myeloma. This was associated with the appearance of a cell clone characterised by a set of traits indicating a low degree of maturity.

Conclusions: We did not find that the type and intensity of cytostatic therapy significantly affect the morphology of plasma cells. Therefore, we suggest that some changes are due to natural, expansive course of the disease.

Key words: plasma cell morphology, morphological classification of multiple myeloma.

Introduction

Multiple myeloma is the most common and most clinically significant disease in the group of plasma cell dyscrasias. This represents about 1.5% of all cancers and about 12% of haematological neoplasia. According to the Polish Myeloma Group, it is estimated that in Poland there are about 1500 cases per year [1].

The treatment of multiple myeloma in the last decade has seen revolutionary change. Before the era of alkylating agents, patients died within the first year. The use of melphalan in the 1960s resulted in improved survival. Another important advance was made in the 1980s, when high-dose chemotherapy and autologous haematopoietic stem cell transplantation were introduced. New drugs such as thalidomide, lenalidomide and bortezomib fundamentally changed the way we treat patients with multiple myeloma. The overall survival of patients in the last decade has improved significantly from 30 months to 45 months [2, 3].

The clinical course of multiple myeloma in individual patients may differ significantly. In chemosensitive disease, patients require intermittent treatment and the use of new forms of therapy can sometimes achieve 10-year survival. The aggressive form of the disease rapidly develops resistance, and patients do not survive one year [4].

This heterogeneity is reflected in the morphology of plasma cells. Multiple myeloma shows extremely high variability in this area. We decided to make a detailed analysis of the morphological characteristics of plasma cells in newly diagnosed myeloma patients, and in the same patients during relapse.

Material and methods

This retrospective study included 103 MM patients, treated with chemotherapy between March 1996 and June 2011 (in a single institution). Median age at diagnosis was 61 years (25–86). The M/F ratio was 1.3. In the study group myeloma isotypes were identified as follows: IgG 69 patients, IgA 20 patients, light chain disease 10 patients, non-secretory multiple myeloma 2 patients, IgD one patient, IgM one patient. Clinical staging was based on Durie-Salmon criteria: 23 patients with stage I, 33 patients with stage II, 47 patients with stage III. Conventional chemotherapy was used in 96 patients. High-dose chemotherapy was used in 7 patients.

Quantitative and qualitative analyses of neoplastic infiltration were based on cytological evaluation of bone marrow smears. Aspiration biopsies were performed by puncturing the sternum or iliac crest. Smears were stained typically with May Grunwald and Giemsa. Preparations had to meet the following conditions:

- satisfactory marrow cellularity;
- percentage of plasma cells at least 10%;
- high-quality staining for the identification of cell types.

For each patient bone marrow cytological preparations obtained from two punctures were evaluated. The first bone marrow biopsy was performed before treatment, at the time of diagnosis. The second bone marrow biopsy was performed at the time of relapse. The interval between these cytological examinations was on average 26 months.

Assessment of morphological and morphometric measurements of myeloma cells was performed using a digital cell imaging system (Olympus). Bone marrow smears were subjected to a three-step evaluation:

First step: Assessment of marrow cellularity in the 40× lens. The smears were reliable with uniform distribution of cells in the viewing field.

Second step: Evaluation of myeloma infiltration was made sequentially counting 500 haematopoietic cells in the 100× lens.

Third step: Evaluation of qualitative changes in myeloma infiltration was made sequentially counting 100 plasma cells in the 100× lens.

The morphological features and morphometric parameters of myeloma cells are shown in Table 1.

Assessment of morphological types of myeloma was based on classifications Goasguen and Greipp [5, 6].

Statistical analysis

For statistical analysis the following were used: Kruskal-Wallis test, Mann-Whitney U-test, Wilcoxon test for non-parametric data, Student's t-test for parametric data.

Results

We found that progression of multiple myeloma was reflected in the alterations of morphology of myeloma cells. The observed differences in some morphological features and morphometric parameters were statistically significant (Table 2).

In recurrent disease myeloma cells are larger, have a larger circuit and increased anisocytosis, increased nucleus surface area and higher nuclear-cytoplasmic ratio. The described changes indicate an increased rate of atypia and cancer malignancy which is usually reflected in the clinical course. In recurrent disease giant plasma cells and flaming cells are more common. In the cytoplasm of myeloma cells the number of vacuoles and Snapper-Schneid granules increases.

Table 1. Morphological characteristics of myeloma cells

Morphological features of myeloma cells	Morphometric parameters of myeloma cells
cell shape	cell surface area
nucleus shape	nucleus surface area
nuclear chromatin structure	nucleoli surface area
number of nuclei	cell diameter long and short
presence and number of nucleoli	cell circuit
content of the cytoplasm and its structure	anisocytosis
presence and distribution of the Golgi apparatus	nucleus diameter
cytoplasmic vacuoles	nucleoli diameter
specific morphological forms: – giant cells – storage cells – signet cells – flaming cells – Mott cellsll – Russell bodies – Dutcher bodies – Snapper-Schneid granules – pseudo-Auer rods	nuclear-cytoplasmic ratio

We evaluated in detail an important prognostic parameter which is the presence of plasma cells with irregular nuclei (PCIN). Earlier studies have shown that PCIN ≥ 5% of marrow plasma cells are a strong, β2-microglobulin-independent, unfavourable prognostic factor (Fig. 1).

Seventeen patients with PCIN ≥ 5% with newly diagnosed multiple myeloma were identified. 25 patients with PCIN ≥ 5% with recurrent disease were identified. Only one patient had lost this morphological feature during treatment.

We analysed the cell morphology in various periods of disease progression. In clinically advanced disease (period III) plasma cells were larger, with more nucleoli and higher nuclear-cytoplasmic ratio.

In the last step we performed a morphological classification of myeloma patients. Two systems, Greipp and Goasguen,

Table 2. Changes in the morphology of myeloma cells

Feature tested	Average value		Significance
$n = 103$	Newly diagnosed	Relapsed	level p
cell surface area (μm²)	202	229	< 0.001
cell circuit (μm)	58	61	< 0.001
anisocytosis*	64	83	< 0.001
nucleus surface area (μm²)	67	82	< 0.01
nuclear-cytoplasmic ratio	0.592	0.623	< 0.001
giant cells (%)	0.82	2.22	< 0.05
flaming cells (%)	2.89	6.35	< 0.01
cytoplasmic vacuoles (%)	26	31	< 0.001
Snapper-Schneid granules (%)	0.22	1.43	< 0.01

**Anisocytosis is presented as the standard deviation of cell surface area.*

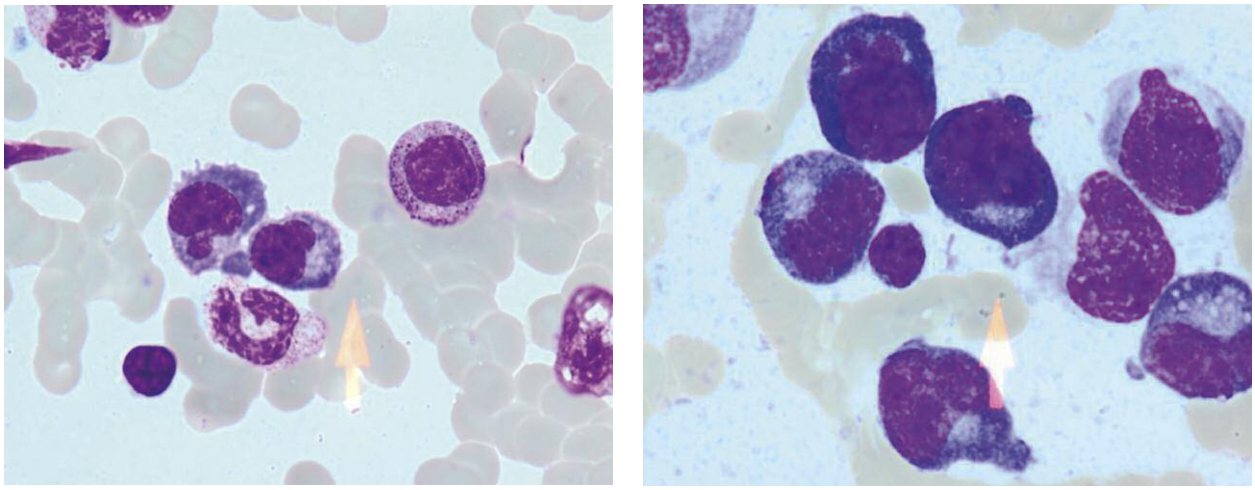

Fig. 1. Myeloma cells displaying irregular nuclear shape. Giemsa stained bone marrow smears. Magnification 100×

Table 3. Morphological types of myeloma cells

Classification	Greipp				Goasguen		
Morphological type	mature	intermediate	immature	plasmablastic	I	II	III
Number of patients (newly diagnosed)	37	38	18	10	51	39	13
Number of patients (recurrent disease)	30	40	16	17	41	42	20

were used. We found that with the biological course of the disease, in some patients there is a change in morphological type of multiple myeloma. We found that the change was noted in 26 patients according to Greipp criteria and in 21 patients according to Goasguen criteria. In most cases change in the morphological type was related to the appearance of a cell clone with a lower degree of maturity (Table 3).

Discussion

Multiple myeloma according to the WHO classification is separated as a distinct disease entity. However, various prognostic factors and results of clinical trials indicate the heterogeneity of the population of patients with this malignancy. Chromosome aberrations discovered in recent years have allowed haematologists to differentiate various groups of patients with significantly different prognosis [7, 8]. Now the question is whether this discrepancy demonstrated by clinical-laboratory tests and cytogenetic studies is associated with polymorphism of myeloma cells viewed under a light microscope [9].

In the available literature we did not find publications that in such detail discussed the morphology of myeloma cells and made assessment at various periods of the disease.

Here we found that in relapsed multiple myeloma, plasma cells are larger, have a larger nucleus and higher nuclear-cytoplasmic ratio, and the anisocytosis is more severe. These features of cell atypia are more pronounced in patients with clinically advanced disease (period III Durie-Salmon). Loss of oval shape of the nucleus is a cytological criterion of malignancy in solid tumours. In multiple myeloma, this feature has already been studied in the 1990s and has been recognized as a strong, poor prognostic factor. Most authors recognize that plasma cells with irregular nuclei significantly affect the prognosis when present in an amount of at least 5% of myeloma cells [10, 11].

Leleu studied 169 patients with newly diagnosed multiple myeloma and 48 patients in relapse. Plasma cells with irregular nuclei ≥ 5% was found in 35/169 newly diagnosed patients (20.7%). Plasma cells with irregular nuclei ≥ 5% was found in 15/48 relapsed patients (31%). Similar results were obtained by Zandecki. PCIN ≥ 5% was found in 34/193 newly diagnosed patients (17.5%). Plasma cells with irregular nuclei ≥ 5% was found in 19/47 relapsed patients (40%). Both Leleu and Zandecki showed that all patients with PCIN ≥ 5% before treatment maintained this feature during relapse.

Cytogenetic studies have shown the relationship between the frequency of plasma cells with irregular nuclei and hypoploidy. Hypoploidy was found in 52% of patients with PCIN ≥ 5% and 21% of patients with PCIN < 5%.

The authors demonstrated significantly different median survival between patients with PCIN ≥ 5% and with PCIN < 5%: 21 months vs. 41 months. Occurrence of PCIN was not correlated with serum 2-microglobulin [10, 11].

In our study, plasma cells with irregular nuclei, regardless of their percentage, were observed in about half of patients, 50/103 (48%). A similar result was reported by Leleu. Before treatment we found PCIN ≥ 5% in 17/103 patients (17%). In recurrent disease we found PCIN ≥ 5% in 25/103 patients (24%). 16 patients with PCIN ≥ 5% with newly diagnosed multiple myeloma maintained this morphological feature despite cytostatic treatment. PCIN incidence was not related to the number of treatment lines, type of treatment (con-

ventional vs. high-dose) or renal function. Furthermore, we found differences in the incidence of PCIN depending on the isotype of monoclonal protein: 5/20 (25%) patients with IgA myeloma vs. 10/69 (14%) patients with IgG myeloma. It seems that it may be responsible for a more aggressive course of disease in patients with IgA myeloma, which is indicated by some authors.

Plasma cells with irregular nuclei are a strong, β2-microglobulin-independent, unfavourable prognostic factor. Assessment of this morphological feature is simple and can be used in clinical practice. PCIN ≥ 5% are observed only during the proliferative process. In monoclonal gammopathy of undetermined significance PCIN never exceed 5%. It appears that PCIN level correlates with an intrinsic factor determining malignancy, rather than a tumour mass [10, 11].

Many studies have confirmed the relationship between the morphology of myeloma cells and the course of disease. Various morphological classifications have been proposed describing the relationship between cell morphology and prognosis. Clinical studies have confirmed that poor prognosis is associated with the occurrence of morphological changes in the nucleus: nuclei size, nuclei shape, number of nucleoli [12, 13]. In 1948 Byard reported that none of ten patients with plasmablastic multiple myeloma survived one year. The plasmablastic type was later identified as a strong, independent, unfavourable factor [6, 14].

In our study, we needed two morphological classifications of multiple myeloma. Plasmablastic type (according to Greipp criteria) and type III (according to Goasguen criteria) were significantly more frequent in recurrent disease.

In conclusion,the variability of morphological forms of myeloma cells may be due to their unique ability to produce antibodies. A collection of immunoglobulins affects the abundance of cytoplasm, changing its structure and staining. Secretory failure results in the formation of vacuoles and various size inclusion bodies. Changes in the cell nucleus – size increase, shape irregularity, diffuse chromatin structure and the presence of nucleoli – are markers of malignancy. In our view changes in the morphology of myeloma cells arise from the natural, progressive course of the disease rather than the applied treatment.

The authors declare no conflict of interest.

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