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CLINICAL RESEARCH

Clinical-Laboratory Significance of Myelofibrosis in Patients with Multiple Myeloma

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Abstract

Background: Currently, there are not many studies of correlations between myelofibrosis (MF) and clinical-laboratory data on multiple myeloma (MM).

Methods and Results: In our study, MF was evaluated according to the scale of the European consensus (2005). Additionally, we used an automated morphometric study. The inverse correlations between the prevalence of MF and the total number of erythroid cells and megakaryocytes, as well as hemoglobin level were found. The total relative area of fibrosis tissue (Srel.fibr.tis.) in initial and advanced MF>20% was associated with anemia requiring a blood transfusion.The development of severe anemia was observed in patients with a greater relative area of Srel.fibr.tis. The direct correlations between Srel.fibr.tis. and relative area of tumor tissue (Srel.tum.tis.), between Srel.fibr.tis. and the level of total serum protein, between Srel.fibr.tis. and daily proteinuria were found both in initial and in advanced MF. Additionally, a direct correlation between Srel.fibr.tis. and the number of plasma cells was revealed in initial MF. Greater Srel.fibr.tis. in initial and advanced MF was found in patients with chronic renal failure.

Conclusion: The clinical-laboratory significance of MF in MM is an inhibition of erythroid cells and megakaryocytes and development of anemia. The relative area of fibrous tissue is a marker of tumor volume and tumor progression.

Keywords: *myelofibrosis (MF); multiple myeloma (MM); anemia; tumor progression.*

Introduction

Currently, the role of MF in patients with MM is explored actively. Advanced MF directly correlates with the number of poorly differentiated plasma cells, which by themselves are poor prognostic markers. The literature suggests that simultaneous morphological study of tumor cells and MF during monitoring of MM can provide better prognostic significance than an isolated study of tumor substrate [1].

Patients with increased fibrosis of the bone marrow also had a median survival time of just 11 months [2-4]. Patients with resistant or progressive disease had permanent elevated levels of serum procollagen III aminoterminal propeptide, which is considered as the marker of fibrogenesis. In most patients with responsive disease, serum procollagen III aminoterminal propeptide is normalized, and no relapses were observed in patients who had normal levels of serum procollagen III aminoterminal propeptide [5].

Despite the attention to the prognostic significance of MF in patients with MM, there aren't many studies of correlations between MF and blood-forming tissue and between MF and disease severity, or of associations between MF and anemia.

Therefore, the purpose of our work was to evaluate the associations between different clinical and laboratory signs of MM, to determine the indicators of MF, which are considered as the markers of clinically significant changes.

Material and Methods

We examined 42 patients with MM who were treated in Novosibirsk State Regional Hospital from 2006 to 2012. Informed consent was obtained from each patient. Patients were divided into three groups: Group 1 - initial presentation of the disease (before chemotherapy), Group 2 - response to chemotherapy (response to treatment is not less than the minimum), Group 3 – relapse (before the next line of chemotherapy). We regarded initial presentation of the disease

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and relapse of MM as the active phase of the disease. Patient characteristics are shown in Table 1. Nine people among Group 2 patients received the 1st line therapy, and five people from this group received the 2nd line therapy. Five people among Group 3 patients received the 1st line therapy, and two people from this group received the 2nd line therapy. The treatment was carried out under schemes containing at least two of the following drugs: melphalan, prednisone, cyclophosphamide, bortezomib, dexamethasone.

Table 1.

Clinical characteristics of patients with MM

Variable	Group 1 (n=21)	Group 2 (n=14)	Group 3 (n=7)
Male	8/38.1%	6/42.9%	2/28.6%
Female	13/61.9%	8/57.1%	5/71.4%
Mean Age:			
Men	66.75±3.18	60.33±4.72	65.0±6.0
Women	58.08±3.43	53.25±2.23	63.20±2.75
MM Stage by the Durie-Salmon Staging System:			
I	7	2	1
II	6	7	3
III	8	5	3

We used standard criteria [6], Russian clinical practice guidelines for the diagnosis and treatment of lymphoproliferative diseases[7], as well as the Durie-Salmon criteria[8], ISS [9], the criteria of the European Group of Blood and Transplant Bone Marrow - EBMT [10] to verify the diagnosis, staging, and treatment evaluation of MM. Chronic renal failure (CRF) was diagnosed according to the Durie-Salmon criteria [8].

Methods for MF evaluation

MF degree was evaluated according to the scale of the European consensus [11]. MF of the 1st degree (MF-1) was regarded as an initial fibrosis; MF of the 2nd and the 3rd degree as an advanced fibrosis [12].

An automated morphometric study of MF was performed using an image analysis software (AxioVision 4.6), camera (Axio Cam), and microscope (Zeiss). The absolute area of fibrous tissue was measured in paraffin-embedded trephine bone marrow biopsies, impregnated with silver by the method of Gomory and stained by the method of van Gieson with magnification 200. The thickness of paraffin-embedded trephine bone marrow biopsies was 4μm. The absolute area of hematopoietic tissue and the absolute area of tumor tissue were measured in slices of marrow stained with H&E. We studied all the bone marrow cavities of one slice of marrow, which corresponds to 5–7 fields of view of the microscope and 20–30 camera view fields (depending on the size of the slice marrow). The area of one field of view was 364000 μm^2 (0.7 mm^2). The area of fibrous tissue was evaluated with respect to hematopoietic tissue. The absolute total area of fibrous tissue (Sfibr.tis.) within the same slice was obtained by adding the area of fibrous tissue in all fields- of-view of the camera. The absolute total area of hematopoietic tissue (Shematopoet. tis.) was calculated in an analogical way, then the relative area of fibrous tissue was calculated (Srel.fibr.tis.) by the formula Srel. fibr.tis.= Sfibr.tis. / Shematopoet.tis. · 100%. The area of tumor tissue was evaluated relative to the normal hematopoietic, bone, and adipose tissues. The relative area of tumor tissue (S rel.tum.tis.) was calculated by the formula Srel.tum.tis. = Stum.tis. / (N · 364 000) · 100%, where Stum.tis. is absolute total area of tumor tissue, n – the number of analyzed fields of view, 364000 – the area of one field of view.

Data of hemograms and myelograms were used for the study of quantitative indicators. The definition of indicators was performed on hematology analyzer Sysmex XT-2000i. Hemogram included a number of erythrocytes, platelets, granulocytes and lymphocytes, the level of hemoglobin and hematocrit. Myelogram included the number of erythroid, megakaryocytic, granulocytic, lymphoid cells and plasma cells, cells of bone marrow microenvironment: reticular cells, osteoblasts, fibroblasts, adipocytes, adventitial cells, endothelial cells, macrofages. Smears of bone marrow were stained with Romanovsky-Giemsa.

Statistical analysis was performed using the *software* package SPSS (version 17.0). *The mean (M)* and standard deviation (SD were deduced. (Standard Deviation –SD). The Mann-Whitney (U Test) was used to compare the differences between the two independent groups (for nonparametric data). Pearson's Correlation Coefficient (r) was used to determine the strength of the relationship between the two continuous variables. Spearman's rank correlation coefficient was also used. A value of $P<0.05$ and odds ratio (OR)>1.0 were considered statistically significant.

Results and discussion

There was an inverse correlation between Srel.fibr.tis. in initial MF and the total number of erythroid cells (r=-0.037, P=0.813), between Srel.fibr.tis. in advanced MF and the total number of erythroid cells (r=-0.496, P=0.031) in the active phase of MM. Additionally, a negative correlation between total Srel.fibr.tis. of initial and advanced MF and the number of megakarycytes was found in the active phase of MM (r=-0.411, P=0.046).

There was a negative correlation between Srel.fibr.tis. in advanced MF and hemoglobin levels (r=-0.420, p=0.047) in anemic patients in initial presentation of MM. Additionally, a negative correlation between Srel.fibr.tis. in advanced MF and the number of erythrocytes in peripheral blood, between Srel. fibr.tis. in advanced MF and hematocrit (r=-0.560, P=0.030) was found.

Univariate analysis showed that total Srel.fibr.tis. in initial and advanced MF>20% was associated with anemia requiring a blood transfusion (hemoglobin <75 g/l, P=0.049, OR=1.62; CI=0.50-7.15).

The greatest Srel.fibr.tis. in initial (22.50±31.82%) and advanced (25.0±2.83%) MF are observed in patients with severe anemia (P<0.001).

Study of the interrelationship of bone marrow microenvironment cells (BMMC) and hematopoietic cells in the bone marrow smears revealed an inverse correlation between the total number of erythroid cells and the number of

macrophages in the relapse of MM (r=-0.999, P=0.025).

A direct correlation between the number of megakaryocytes and macrophages was revealed both in active phase (r=0.643, P=0.033), and in the phase of the response to treatment (r=0.864, P=0.012). Interactions between the BMMC and the lymphoid and granulocytic germ cells were not identified.

We did not find correlations between the number of fibroblasts and the number of the erythroid, granulocytic, lymphoid and megakaryocytic germ cells, nor did we find a relationship between the BMMC and peripheral blood cells, between the BMMC and hemoglobin level.

The correlations and associations of the prevalence of initial and advanced MF with such markers of tumor mass and tumor progression as Srel.tum.tis. and the number of plasma cells in the myelogram were analyzed.

The relative area of fibrous tissue in initial MF and Srel. fibr.tis. in advanced MF directly correlates with Srel.tum. tis. (r=0.390, P=0.040 in initial MF and r = 0.390, P=0.040 in advanced MF). Results of multivariate analysis showed that initial MF was associated with the presence of tumor lesions of the bone marrow (P=0.036, OR=1.10; CI=1.91-45.58). A direct correlation between Srel.fibr.tis. in initial MF and the number of plasma cells in myelogram was revealed (r=0.577,P=0.005).

The relative area of fibrous tissue in initial MF (17.25±23.71%) and the total Srel.fibr.tis in initial and advanced MF (24.44±25.37%) in the presence of CRF were significantly higher (P<0.05) than analogical indicators in the absence of CRF(2.60±6.30% and 6.18±9.68% respectively).

A direct correlation between the level of total serum protein and Srel.fibr.tis. in initial MF was established (r=0.374, P=0.032). Additionally, a direct correlation between the level of daily proteinuria and Srel.fibr.tis. in initial MF was found (r=0.330, P=0.046).

We found a negative correlation between Srel.fibr.tis. and the total number of erythroid cells in the active phase of MM, probably due to intensive production of growth factors and cytokines in the process of tumor growth, causing the overproduction fibers of stromal microenvironment.

There was demonstrated in a murine model, that the active phase of MM is associated with a higher number of tumor-associated fibroblasts than is the inactive phase (response to chemotherapy). Tumor-associated fibroblasts produce excessive amounts transforming growth factor-β, interleukin-6, insulin-like growth factor-1, vascular endothelial growth factor and fibroblast growth factor-2. Activated tumor-associated fibroblasts enhance chemotaxis, adhesion, proliferation, and resistance to apoptosis in MM cells, contributing to disease progression. In turn, MM cells enhance the proliferation of tumor-associated fibroblasts [13].

Because bone marrow fibroblast is a key cellular mediator of MF, producing factors that increase the growth of fibers of stromal microenvironment [14], as well as cytokines, which suppress erythropoiesis [15], it becomes obvious that there is an inverse correlation between Srel.fibr.tis. and the total number of erythroid cells.

Similar mechanisms, relating to erythroid cells, lie at the basis of the correlation between Srel.fibr.tis. and the number of megakaryocytes. Cytokine inhibition of erythropoietin production also impaired the megakaryocytopoiesis [15].

More likely, the association of total Srel.fibr.tis. in initial and advanced MF > 20% with hemoglobin < 75g/l is a consequence of displacement of hematopoietic cells, as described in the primary MF [14].

Mechanical displacement of hematopoietic cells is carried out as fibrotic fibers and tumor tissue [17]. Moreover, these two factors are interdependent, as shown in our study. So, Srel.fibr.tis. both in initial MF and in advanced MF directly correlates with Srel.tum.tis, Srel.fibr.tis. in initial MF directly correlates with the number of plasma cells.

The development of severe anemia is marked in patients with greater Srel.fibr.tis. Pathogenetic mechanisms of this phenomenon are the same ones associated with an imbalance of cytokines and mechanical displacement of hematopoietic cells, which underlies the formation of anemia in chronic disease [18].

The data of myelogram showed no relationships between the number of fibroblasts and total number of erythroid cells. Furthermore, no correlations between the number of fibroblasts in bone marrow smears and peripheral blood cell counts were found. This fact is probably due to the transformation of fibroblasts into myofibroblasts and matrix-producing cells in the MF development [19].

Observed correlations between the number of macrophages and the total number of erythroid cells have not been described in clinical studies. These results testify to the complexity of the relationships between different BMMC groups and require further study.

A direct correlation between Srel.fibr.tis. and the level of total serum protein, between Srel.fibr.tis. and daily proteinuria indicates the relationship between the tumor mass and MF, as total serum protein and daily proteinuria are markers of tumor volume. Greater Srel.fibr.tis. in initial and advanced MF in CRF patients compared with patients without CRF reflects the relationship of tumor growth and prevalence of MF, because CRF is a marker of tumor progression [20].

Conclusion

Thus for the first time, in this paper we show an inverse correlation between the prevalence of MF and the number of erythroid cells and megakaryocytes, between the prevalence of MF and hemoglobin level. We found that total Srel.fibr.tis. in initial and advanced MF>20% is associated with anemia requiring a blood transfusion. The development of severe anemia was marked in patients with greater Srel.fibr.tis.

The results of the study suggest that the clinical and laboratory significance of MF in patients with MM is the suppression of the erythroid and megakaryocytic germs, progression of anemia.

Direct correlations between Srel.fibr.tis. and Srel. tum.tis., Srel.fibr.tis. and the number of plasma cells in the myelogram, Srel.fibr.tis. and the level of total serum protein, between Srel.fibr.tis. and daily proteinuria, as well as the high Srel.fibr.tis. in initial and advanced MF in CRF patients,

compared with patients without CRF, permit consideration of Srel.fibr.tis. as a marker of tumor volume and progression.

Competing interests

The authors declare that they have no competing interests.

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