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Incidence of Myelofibrosis in Chronic Myeloid Leukemia, Multiple Myeloma, and Chronic Lymphoid Leukemia during Various Phases of Diseases

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Pathomorphological study of trephinobiopsy specimens from 129 patients with lymphoproliferative and myeloproliferative diseases was carried out over the course of chemotherapy. Combinations of initial and manifest myelofibrosis (loose network of reticulin fibers and extensive network of reticulin and collagen fibers, respectively) predominated at the debut of chronic myeloid leukemia, chronic lymphoid leukemia, and multiple myeloma. Manifest myelofibrosis was detected in patients with chronic myeloid leukemia without hematological response (failure of normalization of hematological values) and in patients with progressing and relapsing multiple myeloma. Combinations of foci of initial and manifest myelofibrosis were most incident in patients with progressing and relapsing chronic lymphoid leukemia. The incidence of myelofibrosis was higher in patients with multiple myeloma and chronic lymphoid leukemia progression and relapses and in patients with chronic myeloid leukemia without hematological response than at the disease debut and in case of response to chemotherapy. The response to chemotherapy in patients with chronic myeloid leukemia and chronic lymphoid leukemia was associated with a decrease in the incidence of myelofibrosis. In patients with multiple myeloma responding to chemotherapy, the incidence of myelofibrosis did not change in comparison with the disease debut.

Key Words: *myelofibrosis; chronic myeloid leukemia; multiple myeloma; chronic lymphoid leukemia; trephinobiopsy specimens*

The prevalence of hematological malignancies, including chronic myeloid leukemia (CML), multiple myeloma (MM), and chronic lymphoid leukemia (CLL) is still increasing over the world [5]. The role of myelofibrosis (MF) in these diseases is in the focus of attention [2-4].

MF is a prognostic factors in CML, MM, and CLL [12,13], which necessitates the development of new approaches to evaluation of the qualitative and quantitative parameters of MF. Description of MF should include, along with common parameters, such as the incidence and qualitative composition of fibers (reticulin or collagen) [14], dissemination of the process (*e.g.*, relative area of fibrous tissue in the trephinobiopsy specimens [7].

Comparative characterization of these parameters in lymphoproliferative and myeloproliferative diseases is essential for better understanding of the pathogenesis of hematological malignancies.

We compared the incidence of MF in CML, MM, and CLL during the course of chemotherapy.

MATERIALS AND METHODS

Pathomorphological studies of trephinobiopsy specimens were carried out in 129 patients with CML,

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MM, and CLL (treated at State Novosibirsk Regional Clinical Hospital in 2006-2013) during the course of chemotherapy. The mean age of patients was 59.4±2.3 years. The diagnoses of CML, MM, and CLL, identification of the disease stage, and evaluation of the treatment results were carried out according to the standard criteria [1,5,6,8,9]. The study was approved by the Bioethical Committee of the Institute of Molecular Pathology and Pathomorphology.

In CML group, 28 patients were at the debut of chronic phase, 19 of these with optimal or suboptimal response to chemotherapy. The mean duration of the disease from diagnosis to loss of hematological response was 3.4±1.2 years.

In the MM group, 21 patients were at the debut of the disease, 14 of these with response to chemotherapy (at least minimum), in 7 of these, the disease progressed and relapsed later. The mean disease duration from the debut to relapse or progress was 1.2±0.6 years.

In the CLL group, 80 patients were at the disease debut, 52 of these responded to chemotherapy (partial or complete remission) and in 28 patients, the disease progressed or relapsed. The mean duration of the disease from diagnosis to relapse/progress was 2.6±0.9 years.

The number of CML patients responding to therapy was virtually the same after one and two chemotherapy lines. The majority of patients (12) exhibited loss of hematological response after one chemotherapy line (hydroxycarbamide+IFNα-2β) and three patients lost the response after two lines (hydroxycarbamide+IFNα-2β, imatinib mesilate).

In the CLL group, $^{2}/_{3}$ of patients received one chemotherapy line during the phase of response to chemotherapy and $^{1}/_{3}$ received two lines. A greater number of patients developed relapses after one chemotherapy line, a lesser number — after two lines. The therapeutic protocols were as follows: rituksimab+cyclophosphamide+vinblastin+prednisolone, cyclophosphamide+fludarabin, rituksimab+fludarabin +cyclophosphamide.

In the MM group, of the 14 patients with response to therapy $^{2}/_{3}$ received one chemotherapy line and $^{1}/_{3}$ received two lines. Seven patients with the disease relapses had a history of no more than two chemotherapy lines. The treatment protocols were as follows: melfalan+prednisolone, bortesomib+melfalan+prednisolone, bortesomib+dexamethasone, and bortesomib+cyclophosphamide+dexamethasone.

The severity of MF was evaluated on 4-μ paraffin sections of trephinobiopsy specimens of the ileac bone impregnated with silver by the Gomori method and stained by the van Gieson method, according to the European Consensus Score [14].

The results were statistically processed using SPSS 17.0 software.

RESULTS

Evaluation of the qualitative and quantitative parameters of MF showed initial fibrosis (degree 1 MF) and manifest fibrosis (degrees 2 and 3 MF) [12]. The initial MF in all studied lymphoproliferative and myeloproliferative diseases was characterized by the appearance of numerous reticulin (argyrophilic) fibers in the bone marrow forming a network clearly visualized by Gomori staining (Fig. 1, *a*). The progress of MF was associated with accumulation of reticulin fibers forming foci in some cases; in addition, collagen fibers appeared and formed small bundles. Manifest MF was characterized by predominantly thick bundles of collagen fibers occupying large areas in the bone marrow (Fig. 1, *b*).

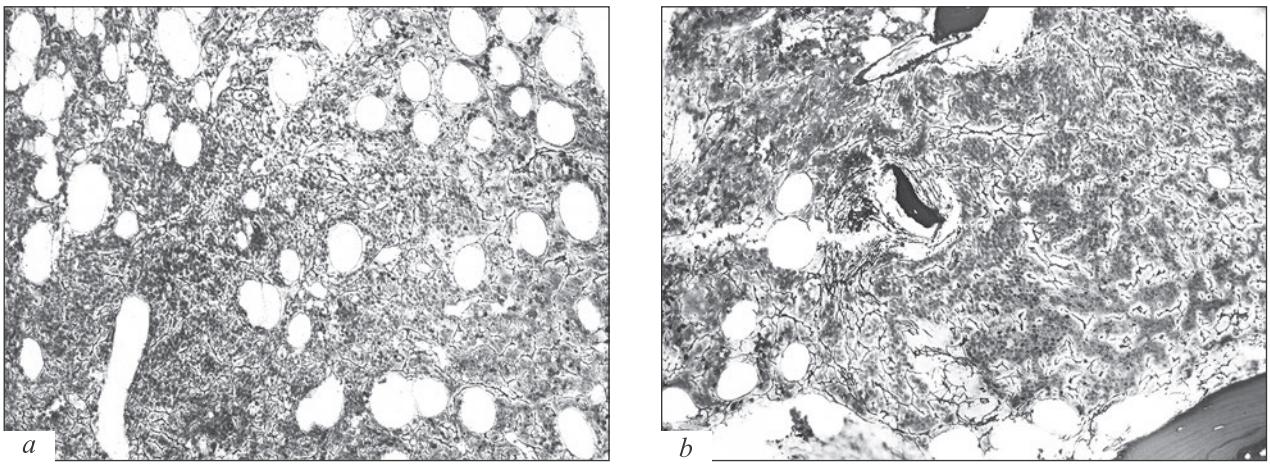

Fig. 1. Chronic lymphoid leukemia. Debut. Trephinobiopsy specimen of the ileac bone. Silver impregnation after Gomori, ×250. *a*) Initial MF; *b*) combination of initial and manifest MF.

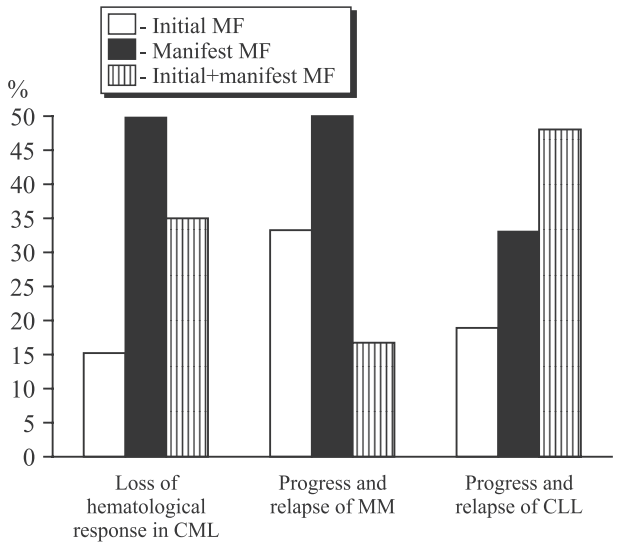

Fig. 2. Structure of MF in CML patients with lost hematological response, in MM and CLL progression and relapses.

The incidence of MF was maximum in patients with MM during all phases and minimum in patients with CLL (Table 1). In CML patients, MF was detected mostly in cases with lost hematological response (p=0.012), while in CLL patients MF was more often associated with the disease relapse and progress (p=0.046). The incidence of MF in CML and CLL was minimum in case of response to chemotherapy (p<0.05). The incidence of MF during MM debut was the same as during response to chemotherapy.

The debut of CML and CLL was associated with combinations of initial and manifest MF foci (46.0 and 44.4%, respectively; p<0.05; Table 2). The debut of MM was characterized by about the same incidence of initial and manifest MF foci and their combinations.

Manifest MF predominated under conditions of hematological response loss in CML and in MM progress and relapses (p<0.05; Fig. 2). Combined initial and manifest MF predominated in CLL patients with the disease progress and relapses (p<0.05). Initial MF was quite rare in CML patients with hematological response loss and in CLL patients with the disease progress and relapse (p<0.05).

Combinations of the initial and manifest MF predominated in the MF structure in patients with chronic CML phase with response to chemotherapy, similarly as in MM patients (p<0.05; Fig. 3). Patients with CML exhibited no initial MF during response to chemotherapy, while CLL patients exhibited no manifest MF during this phase. In MM patients the initial MF was more incident during response to chemotherapy than manifest MF (p=0.037).

One-way analysis detected an association of manifest MF with CML duration over 3 years (p=0.016, OR=69.05, 95%CI 2.45-417.10) and with MM duration over 2 years (p=0.003, OR=75.12, 95%CI 4.78-350.13). No association of the initial MF with the duration of CML, MM, or CLL was detected.

TABLE 1. Incidence of MF (%) in CML (Chronic Phase), MM, and CLL over the Course of Chemotherapy

Disease		Incidence of MF, %
CML (chronic phase)	debut	46*
	loss of hematological response	52**
	response to chemotherapy	32***
MM	debut	57°
	progress and relapse	85.7°°
	response to chemotherapy	57°°°
CLL	debut	22.5*
	progress and relapse	39.0**
	response to chemotherapy	28.9***

Note. p<0.05 in comparison with MF incidence *at MM debut, **in MM progression and relapse, ***in response to chemotherapy in MM, °at CLL debut, °°in CLL progression and relapse, °°°in response to chemotherapy in CLL.

Our study demonstrated different incidence of MF in various phases of CML, MM, and CLL, which can be explained by peculiarities of pathogenesis, chemotherapy protocols, and disease duration [2,3,12]. Combinations of initial and manifest MF foci predominated at the debut of CML and CLL. The debut of MM was characterized by equal incidence of the initial and manifest MF and their combinations. Our data indicated parallel development of the initial and manifest MF from the very beginning of disease. A longer course of CML and MM was associated with manifest MF. Presumably, this fact can be explained by longer persistence of tumor cells directly contributing to the fiber production by fibroblasts due to the growth factors they synthesized. The concentrations of proinflammatory cytokines with angiogenic and fibrogenic activities increased with the diseases progress [10,11].

TABLE 2. Structure of MF (%) during the Debut of CML, MM, and CLL

MF	CML debut	MM debut	CLL debut
Initial MF	25	33.3	33.3
Manifest MF	29	33.3	23.3
Initial+ manifest MF	46	33.3	44.4

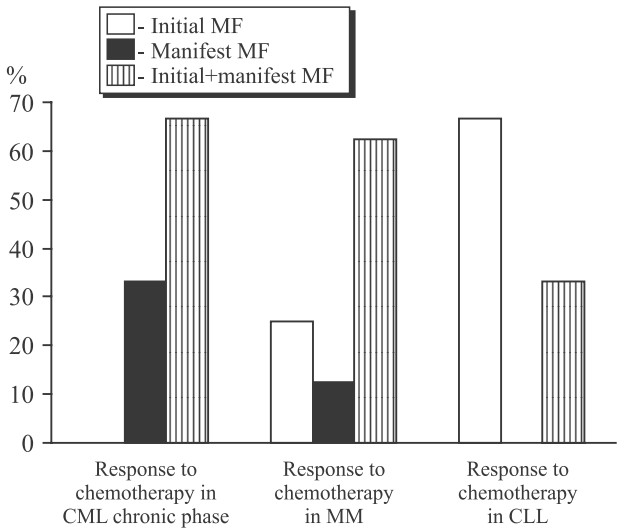

Fig. 3. Structure of MF in chronic phase of CML, in MM and CLL during response to chemotherapy.

Manifest MF predominated in MM progress and relapse and in CML patients with lost hematological response. The progress and relapse of CLL were characterized by mainly combined foci of initial and manifest MF. Relapses and progression of CLL and MM and lost of hematological response in CML were characterized by a higher incidence of MF in comparison with the debut and the response to chemotherapy phase, presumably due to the summary effects of the tumor and chemotherapy on fibrogenesis [4].

Combinations of initial and manifest MF foci predominated in the MF structure in CML patients with response to chemotherapy; the incidence of MF decreased during therapy. In MM patients responding to chemotherapy the combinations of initial and manifest MF foci were the most incident; the incidence of MF in patients responding to therapy was the same as during the disease debut. The incidence of MF decreased in CLL patients during response to chemotherapy, the initial MF predominating in the MF structure.

The decrease in the count of tumor clone cells and their disappearance as a result of chemotherapy led to MF regression in the majority of cases. This MF regression could be caused by a decrease in the count of tumor cells, changed quantitative and qualitative proportions of the bone marrow microenvironment cells, and (in CML) by a decrease in megakaryocyte count [2,4]. A lesser incidence of MF detected in our study could be attributed to frequent manifestations of the total systems reaction: fibrous tissue regression, observed in reduction and/or elimination of the etiological factor (agent). The incidence of MF was higher in all phases of MM than in CML and CLL; this fact deserved further studies of MF incidence and dissemination (the area occupied by MF in trephinobiopsy specimens).

Hence, combinations of initial (loose network of reticulin fibers) and manifest (well-disseminated network of reticulin and collagen fibers) MF predominates during the debut of CML, CLL, and MM. Manifest MF is highly incident in CML patients without normalization of hematological parameters (no hematological response) and in MM patients with the disease progress and relapses. Progression and relapsing of CLL are associated with combined initial and manifest MF foci. The incidence of MF is higher in patients with MM and CLL progress and relapses and in CML patients without hematological response than during the disease debut and response to chemotherapy. Response to chemotherapy in CML and CLL is associated with a lesser incidence of MF. The incidence of MF in MM patients responding to chemotherapy does not differ from that during the disease debut.

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