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The spleen in osteomyelofibrosis

A morphological and immunohistochemical study of 30 cases

Stephan Falk, Detlef Mix, and Hans-Jochen Stutte

Department of Pathology, J.W. Goethe University Frankfurt, West Germany

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Summary. 30 spleens from patients with biopsy proven primary osteomyelofibrosis were studied by histological and immunohistochemical methods. The presence of trilinear haematopoiesis along the splenic circulatory pathway supports the theory that the spleen acquires haematopoietic precursor cells by filtration of the peripheral blood. In addition, impairment of intrasplenic circulation with subsequent red pulp congestion, pulp cord fibrosis and haemophagocytosis is of importance for the pathogenesis of both marked splenic haematopoiesis and complications due to hypersplenism.

Key words: Hematopoiesis – Immunohistochemistry – Osteomyelofibrosis – Spleen

Introduction

Osteomyelofibrosis (OMF), also known as osteomyelosclerosis, myelofibrosis or agnogenic myeloid metaplasia (Rappaport 1966; Wintrobe et al. 1981) is a myeloproliferative disorder which is characterized by a leukoerythroblastic blood picture, progressive fibrosis of the bone marrow and a marked extramedullary proliferation of haematopoietic cells (Pitcock et al. 1962; Ward and Block 1971). In most cases massive splenomegaly constitutes the leading clinical sign. Several previous studies have ascribed a major role to the spleen with regard to the pathogenesis and to the typical complications of OMF (Dameshek 1951; Bouroncle and Doan 1962; Niles et al. 1959; Rappaport 1966). The present study examines a large series of splenectomy specimens with conventional histological and immunohistochemical methods in order to analyse the pathogenesis of splenomegaly and spleen-related complications in the setting of OMF.

Offprints to: Stephan Falk, Department of Pathology, J.W. Goethe University, Theodor-Stern-Kai 7, D-6000 Frankfurt 70, Federal Republic of Germany

Materials and methods

30 splenectomy specimens from 19 male and 11 female patients aged 46 to 74 years (median: 62 years) suffering from OMF were studied. In all cases the diagnosis of primary OMF (de novo OMF without any preexisting myeloproliferative disease) had been established prior to splenectomy by examination of a bone marrow biopsy and the presence of a typical leukoerythroblastic blood picture. All but two of the patients had been managed expectantly; the patients had received supportive treatment such as blood transfusions but no alkylating agents or radiation therapy.

All spleens were examined macroscopically with respect to size, weight and gross abnormalities. Three to five tissue blocks from different sites were fixed in 4% formalin and processed for conventional histological examination (HE, PAS, Giemsa, silver and Perl's iron stain). Selected blocks from each case were subjected to immunohistochemical studies using a modified alkaline phosphatase method (Cordell et al. 1984). The primary antibodies used are given in Table 1. In addition, granulopoietic cells were stained with the AS-D-chloroacetatesterase reaction (Leder 1964).

Results

Macroscopic findings

Most of the spleens are excessively enlarged. The weights range between 280 and 5700 g with a mean of 2025 g. The splenic capsule is usually thickened and shows patchy fibrosis, and in most cases the splenic parenchyma appears slightly indurated. Splenic infarcts measuring up to 10 cm in diameter are present in six cases. On the cut surface Malpighian corpuscles are indistinct, while the red pulp appears homogenous and in many instances possesses a brownish hue. In several instances irregular yellowish-brown nodules with a diameter of up to one centimeter corresponding to Gandy-Gamna bodies are present. In four cases nodular lesions of up to 2 cm in diameter are evident.

Table 1. Antibodies used in the present study

Designation	Specificity	Source
Ret 40f	Erythropoietic cells	K.C. Gatter, Oxford
Y2/51	CD61: thrombo-/ megakaryocytes	K.C. Gatter
Neutrophil elastase	Granulopoietic cells	Dako Corp.
UCHL-1	CD45RO: T lymphocytes	Dako Corp.
L26	B lymphocytes	Dako Corp.
Protein S100	Macrophages, reticulum cells	Camon Corp.
BerMacDRC	CD35: dendritic reticulum cells	H. Stein, Berlin
EBM 11	CD68: Macrophages	Dako Corp.
Lysozyme	Granulocytes, monocytes	Dako Corp.
Vimentin	Intermediate filaments	Dako Corp.

Camon Corp.: Camon GmbH, D-6200 Wiesbaden, FRG; Dako Corp.: Dako GmbH, D-2000 Hamburg, FRG

Microscopic and immunohistochemical findings

Histologically, all splenectomy specimens show a marked expansion of red pulp structures. The Malpighian corpuscles are small and contain very few germinal centers in the follicles and narrow marginal zones. Their number is reduced. In most cases they are widely separated by red pulp structures that comprise highly cellular pulp cords and sinus which are either dilated or compressed. The pulp cords appear congested and, especially in very large spleens, exhibit diffuse fibrosis. In many instances there is perifollicular bleeding into the pulp cords which, in later stages, leads to perifollicular fibrosis and siderosis.

Haematopoietic precursor cells are evident both in the pulp cords and in the sinus. In all cases this extramedullary haematopoiesis is trilinear, while the relative contribution of each haematopoietic cell line is variable (Fig. 1).

In 27 cases granulopoietic precursors predominate; these cells, promyelocytes, myelocytes and occasional myeloblasts are mostly located within the pulp cords where they are readily identifiable by their reactivity for neutrophil elastase and naphthol-AS-D-chloroacetatesterase (Fig. 2). Neutrophil precursors constitute the majority of granulopoietic precursor cells; in addition, some eosinophils and basophils are also present. Small numbers of granulopoietic precursors are visible within the sinus. In three cases erythroid cells predominate. As in the other splenectomy specimens, they are most evident in the sinus where normoblasts and proerythroblasts tend to cluster around macrophages in order to form erythron-like structures. However, in many instances irregular and giant erythra are present. Foci of erythroblasts reactive with Ret 40f are also present in the pulp cords. Intrasinusoidal erythropoietic foci are often located in the immediate vicinity of erythroid cells within the pulp cords, and in many instances erythroid cells traverse the sinus wall into the sinus (Fig. 3). In four cases splenic erythropoiesis exhibits marked megaloblastic changes.

The megakaryocytes which also occur both within the pulp cords and the sinus exhibit severe dysplastic features: their nuclei are highly polymorphic and so-called immature megakaryocytes with condensed nuclear chromatin appear. Numerous megakaryocytes appear to migrate from the pulp cords through the sinus walls into the sinus. In all instances they are reactive with Y2/51, an antibody against platelet glycoprotein III (CD61). This antibody also stains numerous thrombocytes some of which are so-called giant thrombocytes. Most thrombocytes are present in the pulp cords where they may be observed to cluster around sinus walls (Fig. 4). In four spleens which are only moderately enlarged and which contain only few haematopoietic cells megakaryocytes are seen to form band-like infiltrates around Malpighian corpuscles (perifollicular infiltrates).

In addition there are numerous macrophages which contain lysozyme, proteinase inhibitors and which are reactive with the EBM11 (CD68) antibody. The majority of these mononuclear phagocytes populate the pulp cords and exhibit marked haemophagocytosis. As a consequence, many macrophages either contain iron granules or are transformed into PAS-positive foam cells. Many macrophages express S-100 protein which also holds true for the macrophages found within erythropoietic foci and some phagocytosing cells within the sinus. In addition, there are increased numbers of S-100-positive interdigitating cells and CD45RO-positive T cells among the haematopoietic foci, while B lymphocytes reactive with the L26 antibody are restricted to the remaining splenic B cell areas. These correspond to follicles with a compressed network of BerMacDRC-positive dendritic reticulum cells.

In six cases marked plasmacytosis of the pulp cords is evident; the plasma cells are mostly observed in the immediate vicinity of trabeculae and pulp veins.

The nodular formations evident on the cut surface in four cases correspond histologically to pseudotumorous aggregates of haematopoietic cells, mostly composed of atypical megakaryocytes with admixed granulo- and erythropoietic precursors and macrophages. In two cases incipient lesions of this type are present. Here, lakes of extramedullary haematopoiesis are visible within dilated pulp cords leading to compression of the adjacent sinus whose endothelial cells are destroyed (Fig. 5). Dilated sinus or compressed venous blood vessels are usually visible within the immediate vicinity of such lesions.

In early stages of splenic involvement of OMF when the spleen is relatively small fiber architecture is largely preserved despite the presence of extramedullary haematopoiesis. These spleens typically do not show signs of impeded intrasplenic circulation. While thickening of the ring fibers may be seen (Fig. 6), marked fibrosis of the pulp cords with irregular and coarse fiber bundles which are reminiscent of the fibrosis seen in bone marrow specimens from patients with OMF is usually only observed in very large spleens (> 3000 g). However, marked fibro-

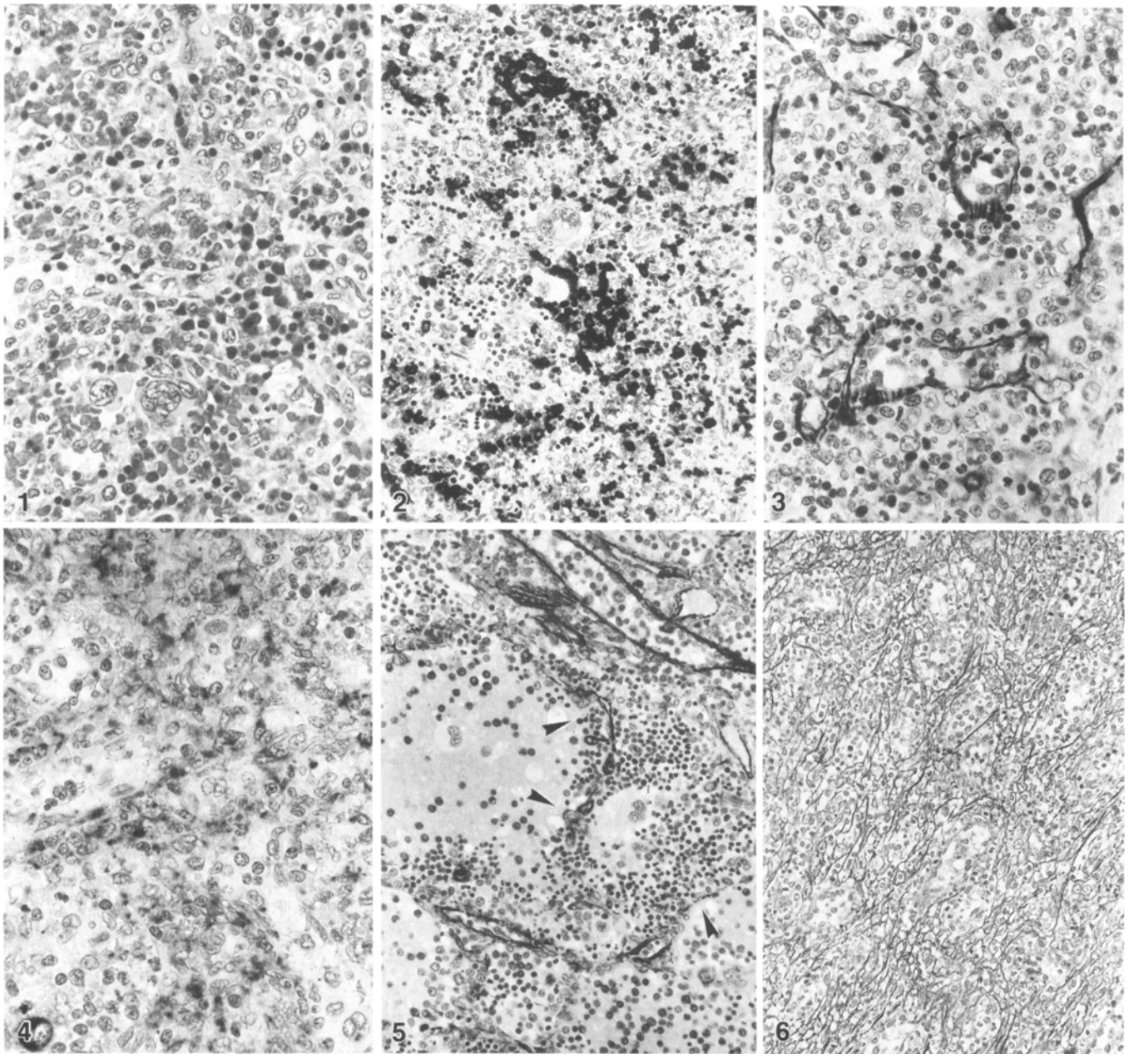

Fig. 1. Spleen in OMF with trilinear extramedullary haematopoiesis. Atypical megakaryocytes and granulopoietic precursors are present in congested pulp cords and sinus. HE, ×310

Fig. 2. Large numbers of granulopoietic cells may be visualized by staining for elastase or for chloroacetate esterase. ASDCL, ×165

Fig. 3. Erythroblasts leave pulp cords distended by haematopoietic cells and pass between vimentin-positive sinus endothelia into the sinus. Vimentin, APAAP, ×310

Fig. 4. CD61-positive thrombocytes line the outer sinus walls and are retained in the pulp cords. An atypical megakaryocyte is also reactive. Y2/51, APAAP, ×310

Fig. 5. Formation of a pseudotumorous nodule composed of haematopoietic cells in distended pulp cords with destruction of adjacent sinus endothelia (*arrows*). Vimentin, APAAP, ×165

Fig. 6. Early in the course of OMF spleens do not exhibit major alterations of the fiber architecture, although some degree of sclerosis may be evident in the pulp cords. Gomori, ×165

Table 2. Important morphological features of the spleen in OMF

- Spleens in OMF are almost always massively enlarged
- Trilinear haematopoiesis is preferentially localized in the pulp cords, but passage of immature blood cells into sinus also leads to sinus involvement
- Amount and composition of haematopoiesis may vary considerably, but atypical megakaryocytes are always present
- Pseudotumorous foci of haematopoietic cells are associated with signs of circulatory disturbances and develop within dilated pulp cords
- During early OMF the splenic fiber architecture is preserved, while in later stages there is diffuse sclerosis of the pulp cords
- Periarteriolar bleeding and scarring as well as other signs of circulatory disturbances are frequent
- In the pulp cords retention and phagocytosis of haematopoietic cells and of thrombocytes may be observed by conventional histological and immunohistochemical stains

sis is absent from pseudotumorous nodules of haematopoietic cells in which megakaryocytes predominate and splenic fibrosis is not associated with the number of megakaryocytes. All spleens with marked pulp cord fibrosis show severe congestion of pulp cords by erythrocytes and thrombocytes as well as compressed venous vessels within the red pulp. In many instances subendothelial infiltrates of haematopoietic cells in trabecular veins are visible and in at least one case these are associated with thrombosis of a trabecular vein. These changes are accompanied by regional ectasia of sinus and regressive changes such as periarteriolar bleeding, formation of Gandy-Gamna bodies and splenic infarcts. The main results are summarized in Table 2.

Discussion

The pathogenesis of splenic haematopoiesis in osteomyelofibrosis has long been a a matter of controversy. Today it is generally accepted that extramedullary haematopoiesis in OMF is composed of neoplastic haematopoietic precursor cells and cannot be regarded as a compensation process for bone marrow failure (Wolf and Neiman 1985). The hypothesis that splenic haematopoiesis results from the activation of dormant haematopoietic stem cells in the spleen (Dameshek 1951; Ward and Block 1971) has been discarded in favor of the so-called filtration theory (Wolf and Neiman 1987, 1989). This theory is supported by the absence of significant fetal haematopoiesis in the spleen and the presence of large numbers of haematopoietic precursor cells in the peripheral blood of OMF patients.

The results of the present study provide further support for the filtration theory. In addition, our findings contribute to the understanding of the pathological processes which lead to the development of massive splenic haematopoiesis.

Most spleens in patients with OMF are rather large; in fact, most of them are removed either for painful splenomegaly or for hypersplenism (Silverstein and Re-Mine 1979). However, it is not exactly known which mechanisms are responsible for the massive enlargement of the spleen in OMF, since splenomegaly is not solely due to splenic infiltration by haematopoietic cells. Our results suggest that while haematopoetic cells are present in large numbers in the pulp cords, passive congestion of the cords by erythrocytes contributes significantly to splenic enlargement. This assumption is in keeping with recent radionuclide scanning studies employing labelled erythrocytes which demonstrated that the increase in splenic size in OMF is due both to an increase in splenic "vascularity" (i.e. splenic erythrocyte and plasma contents) and in "cellularity" (i.e. spleen volume less splenic vascularity; Zhang and Lewis 1989). These results imply that erythrocyte retention and extramedullary hematopoiesis which augment vascularity and cellularity both contribute to the splenomegaly.

Unfortunately the filtration theory does not explain adequately why haematopoietic precursor cells are retained in the spleen despite their ability to pass through pulp cords and sinus. The fact that low level splenic haematopoiesis is a constant finding and may be linked to the presence of haematopoietic precursors in the peripheral blood of haematologically normal individuals (Falk et al. unpublished data) shows that splenic haematopoiesis in OMF may not be attributed solely to a microenvironment suitable for the proliferation of haematopoietic cells. An alternative hypothesis is that neoplastic haematopoietic cells in OMF alter their growth and microenvironmental requirements, thus shifting proliferation activity away from the bone marrow into the spleen. The results of the present study show that splenomegaly increases with duration of the disease and is closely correlated with congestion and fibrosis of pulp cords in accordance with earlier findings (Lewis 1985; Thiele et al. 1989). The demonstration of a pronounced involvement of the pulp cords and the presence of haematopoietic precursor cells in the perifollicular region where most afferent blood vessels terminate (Van Krieken et al. 1985) as well as the passage of immature blood cells through the sinus walls lend credence to the filtration theory. However, the frequent detection of dilated sinus, compressed pulp veins and periarteriolar bleeding and/or fibrosis point to the importance of circulation disturbances in the pathogenesis of massive splenic haematopoiesis. Congestion and concomitant expansion of the pulp cords by immature and mature blood cells will undoubtedly increase intrasplenic circulation time and thus splenic pooling of blood cells (Aster 1966). In fact, recent studies of enlarged spleens in various types of haematological malignancies have established that while the splenic blood flow increases in splenomegaly, splenic perfusion (blood flow in relation to splenic volume) decreases (Peters et al. 1984; Wadenvik et al. 1987). Concomitantly, the splenic platelet pool is expanded (Wadenvik et al. 1987). The retention of haematopoietic cells is best illustrated by the formation of pseudotumors (Goldman and Nolasco 1983) in dilated pulp cords next to compressed pulp veins or periarteriolar areas of fibro-

sis. In addition, haematopoietic cells crowded in pulp cords in the immediate vicinity of the sinus walls without passing into the sinus are observed in many instances. This phenomenon is presumably due to the progressive fibrosis seen in OMF spleens. However, the pathogenesis of splenic fibrosis in OMF appears to differ from the factors responsible for bone marrow fibrosis in OMF. Splenic fibrosis usually appears only in spleens which are already markedly enlarged (Ward and Block 1971; Bouroncle and Doan 1962; Wolf and Neiman 1989). In contrast to bone marrow fibrosis (Thiele et al. 1989) an increase in splenic fibers is not linked to the presence of large numbers of atypical megakaryocytes. This is especially obvious in splenic pseudotumors composed of haematopoietic precursors. Moreover, spleens in OMF usually show a preservation of fiber architecture, while the bone marrow exhibits marked and diffuse fibrosis. These findings could be due to a different reactivity of bone marrow and splenic fibroblasts to cytokines secreted by atypical megakaryocytes (Castro-Malaspina et al. 1981), but this possibility seems rather remote. Instead the lack of fibrosis near large quantities of abnormal megakaryocytes and the preservation of fiber architecture despite the presence of large numbers of fibroblasts in the spleen points to the importance of other factors, notably of impaired intrasplenic circulation.

Most spleens in advanced OMF histologically show the salient features of circulatory disturbances by congestion of the pulp cords and obstruction of venous outflow. These changes are reminiscent of the alterations caused by portal hypertension – a state in which a pronounced splenic haematopoiesis has been identified (Palitzsch et al. 1987). In OMF intrasplenic circulation is impeded by the presence of large numbers of haematopoietic cells. As our morphological and immunohistochemical findings show, these cells predominantly populate the pulp cords, clog the sinus walls and cause compression of pulp veins. Congestion of the pulp cords in turn enhances the retention of haematopoietic precursor cells from the peripheral blood via increased splenic pooling in the slow compartment of splenic circulation. At the same time, it causes periarteriolar bleeding with associated fibrosis and an increase of fibre density in the pulp cords which further impedes the passage of immature haematopoietic cells through the spleen and thus completes the vicious circle.

In addition, the expansion of splenic pulp cords and impairment of intrasplenic circulation may also cause retention of mature blood cells (hypersplenism) – a frequent complication in OMF patients. Its morphological equivalents include splenomegaly with increased pulp cord volume (Aster 1966) and an increase in number of CD68+ macrophages in the pulp cords and sinus which exhibit marked haemophagocytosis (Tavassoli and Weiss 1973). This may be stimulated by intrinsic defects of the neoplastic haematopoietic cells but may also be due to mechanical injury encountered during passage through congested pulp cords and partially blocked sinus walls. The peculiar deformations of erythrocyte shape encountered in OMF may result from such mechanical injuries (DiBella et al. 1974). In addition, the large number of S-100-positive macrophages and interdigitating cells which largely function as antigen-presenting cells suggest that increased amounts of antigenic material are present which presumably result from the breakdown of haematopoietic cells.

In conjunction with the megaloblastic changes and the marked haemophagocytosis mechanical injury may contribute to the ineffective erythropoieisis (Pettit et al. 1976; Szur and Smith 1961) and to the thrombocytopaenia (Wintrobe et al. 1981; Ward and Block 1971) frequently encountered in OMF.

Splenomegaly in OMF is caused by progressive retention of haematopoietic precursor cells which in conjunction with their local proliferation lead to circulatory disturbances with pulp cord fibrosis. These in turn are responsible for increased splenic filtration of haematopoietic cells and of mature blood cells which gives rise to both enhanced splenic haematopoiesis and hypersplenism.

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