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ORIGINAL RESEARCH REPORT

The effects of hematopoietic stem cell transplant on splenic extramedullary hematopoiesis in patients with myeloproliferative neoplasm-associated myelofibrosis

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KEYWORDS
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Abstract
Background/objective: Hematopoietic stem cell transplant (HSCT) is the only curative treatment for myeloproliferative neoplasm-associated myelofibrosis (MPN-MF). The main clinical manifestation of MPN-MF is splenomegaly secondary to extramedullary hematopoiesis (EMH). The effects of HSCT on splenic EMH and associated vascular and stromal changes are unknown. This study compares the findings seen in spleens following HSCT with those of nontransplanted patients, normal controls, and matched bone marrow (BM) samples.
Methods: This study included three transplanted MPN-MF spleens, three nontransplanted MPN-MF spleens, and three normal controls. Spleens were assessed for: (a) presence/extent of EMH; (b) presence of Gamna–Gandy bodies; (c) splenic fibrosis; (d) CD34-positive microvessel density; (e) CD8-positive sinusoids; (f) frequency of smooth muscle actin-positive myoid cells; and (g) nerve growth factor receptor-positive adventitial reticulum cells. In two cases,

Abbreviations: BM, bone marrow; CR, complete remission; ET, essential thrombocythemia; HSCT, hematopoietic stem cell transplant; MF, myelofibrosis; MPN, myeloproliferative neoplasm; PMF, primary myelofibrosis; PV, polycythemia vera; RIC, reduced intensity conditioning

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matched BM samples were assessed for cellularity, presence of atypical megakaryocytes, and fibrosis.

Results: Compared with normal controls, all MPN-MF spleens were larger in size, had EMH, red pulp fibrosis, higher CD34-positive microvessel density, and decreased CD8-positive sinusoids. Compared with nontransplanted cases, post-HSCT spleens showed disappearance or reduction of EMH. Gamna—Gandy bodies were increased; no differences in the remaining parameters were found. A reduction of splenic EMH was associated with normalization of BM cellularity and megakaryopoiesis.

Conclusion: HSCT reduces/abrogates splenic EMH and is associated with an increased number of Gamna—Gandy bodies, which may suggest vascular damage. The lack of stromal changes in spleens removed shortly after transplant is in line with similar observations in the BM, where a longer interval is often necessary for resolution of fibrosis.

Introduction

Primary myelofibrosis (PMF), polycythemia vera (PV), and essential thrombocythemia (ET) are Philadelphia-negative myeloproliferative neoplasms (MPN), which originate from the neoplastic transformation of marrow hematopoietic stem cells [1—3]. Although PMF, PV, and ET are distinct oncological entities, their advanced myelofibrotic stages share several clinicopathological features [4,5] and are collectively referred to as MPN-associated myelofibrosis (MF).

One of the distinct clinical features of MPN-MF is the presence of splenomegaly [6], which is the direct consequence of extramedullary hematopoiesis (EMH), by which proliferating hematopoietic cells expand and efface the splenic architecture [7,8]. Several theories have originally addressed the pathobiology of EMH in MPN-MF: (a) the compensatory theory, considering EMH as a compensatory reaction to extensive bone marrow (BM) fibrosis; (b) the myelostimulatory theory, hypothesizing the reactivation of dormant splenic hematopoiesis in response to MF-derived cytokines; and (c) the neoplastic theory, considering EMH as the splenic localization of neoplastic hematopoietic cells [9]. From the 1980s onward, the neoplastic nature of splenic EMH was definitely proven by its association with cytogenetic abnormalities, presence of clonal X chromosome inactivation, and of *JAK2*^{V617F} mutation [10—15].

It is currently believed that EMH in MPN spleens is associated with abnormal trafficking of BM-derived clonal hematopoietic progenitors and stem cells, due to the dysregulation of the marrow microenvironment. This results in circulating mature and immature marrow elements that home to the spleen. These cells are largely capable of producing the typical histologic manifestation of EMH, that is, trilinear hematopoiesis including erythroblasts, myeloid precursors with a predominance of maturing forms, and megakaryocytes.

Splenic EMH is largely centered on the splenic red pulp, induces marked atrophy of the white pulp, with disappearance of B-cells and reduction of T-cell perivascular cuffs [16—18]. EMH proliferates with a diffuse, nodular, or mixed growth pattern [16,17]. In addition, MPN-MF is capable of affecting both red pulp vessels and stromal cells, with a marked decrease of CD8-positive splenic sinusoids and a parallel increase of CD34-positive microvessels and interstitial fibrosis. These changes were confirmed by the demonstration of an altered distribution of smooth muscle actin (SMA)-positive myoid cells and of nerve growth factor receptor (NGFR)-positive adventitial reticulum cells [16]. Of note, none of these studies included spleens obtained following hematopoietic stem cell transplant (HSCT).

The present study thus aims to describe the histologic changes induced by HSCT in spleens of MPN-MF patients. The morphological and immunohistochemical features of such cases were compared with those of spleens from nontransplanted MF patients and with normal controls. Possible correlations between post-HSCT spleen and BM findings (i.e., cellularity, presence of atypical megakaryocytes, and presence of fibrosis) were also studied.

Materials and methods

Case selection

The present study considered a single Institution series of patients with history of MPN-MF, treated with HSCT. Among 18 patients with an enlarged spleen at the time of HSCT, 14 had documentation of the spleen status after HSCT. Four patients had normalization of the spleen size, seven patients had persistent splenomegaly, and three patients underwent splenectomy due to poor count recovery.

The present study focused on the histologic and immunohistochemical features of the spleens removed from the latter three patients (2 men and 1 woman; age: 55 years, 52 years, and 33 years). Two cases had a diagnosis of PMF and one case had post-PV MF. Pretransplant clinical data (i.e., Lille score, Dynamic International Prognostic Scoring System score, Eastern Cooperative Oncology Group performance status, number of circulating blasts, lactic acid dehydrogenase levels) and post-transplant follow-up were available in all cases. The response to therapy was assessed according to the revised International Working Group-Myeloproliferative Neoplasms Research and Treatment and European LeukemiaNet criteria [19]. BM biopsies after splenectomy were available in two cases.

Data on *JAK2* mutational status were available for both transplanted and nontransplanted MF: three out of six

patients carried the *JAK2*^{V617F} mutation (post-HSCT MF: 1 case; nontransplanted MF: 2 cases); three out of six cases were *JAK2*^{V617F}-negative (post-HSCT MF: 2 cases; nontransplanted MF: 1 case). No information on *MPL* and/or *CALR* mutations was available for the *JAK2*^{V617F}-negative cases.

The morphological and immunohistochemical features of post-HSCT spleens were compared with: (a) three spleens obtained from nontransplanted MF patients (2 men and 1 women; age: 72 years, 71 years, and 63 years), with comparable marrow fibrosis and disease stage (PMF: 2 cases; post-PV MF: 1 case); (2) three normal spleens, removed during major abdominal surgery for nonhematological malignancies (3 men; age: 76 years, 69 years, and 54 years). In both the transplanted and nontransplanted MF subgroups, splenectomy was performed at comparable time intervals after the diagnosis of MPN. In particular, the transplanted patients were splenectomized 4 years, 2 years, and 15 years after the original diagnosis of MPN (mean time interval between diagnosis and splenectomy: 7.0 years). The nontransplanted patients underwent splenectomy 2 years, 4 years, and 23 years after the original diagnosis of PMF or PV (mean time interval: 9.7 years).

Paraffin embedded tissue blocks were retrieved from the archives of the Hematopathology Division of Weill Cornell Medical College, New York, NY. Gross descriptions of each case (weight and size of the spleen and presence of infarcts and/or other focal lesions) were available in all cases.

For each case, appropriate informed consent was obtained. The study was also approved by the Institutional Review Board, consistent with the Declaration of Helsinki.

Histologic evaluation of the spleen

Representative formalin-fixed, paraffin-embedded tissue blocks were selected for histologic evaluation. The following morphological parameters were evaluated: (a) presence and extent of EMH, graded using a 4-tiered score (score 0 = absent; score 1+ = rare clusters of $\leqslant$5 cells; score 2+: either several clusters of 5–10 cells or any clusters of $\sim$50 cells; and score 3+ = numerous small clusters or areas of diffuse involvement) [17]; (b) EMH growth pattern (nodular, diffuse, or mixed, as previously reported) [16]; (c) EMH cellular composition; (d) presence of splenic infarcts; (e) presence of Gamna–Gandy bodies (i.e., sclero-siderotic nodules); and (f) presence of splenic fibrosis (as assessed by reticulin and trichrome stains). Splenic fibrosis was defined as the presence of reticulin/collagen fibers exceeding what was observed in normal controls (i.e., fine fibers around the splenic vessels and red pulp sinusoids).

For the comparison between post-HSCT spleens and BMs, the following marrow parameters were considered: (a) BM cellularity; (b) presence of atypical megakaryocytes (i.e., megakaryocytes with large, bulbous nuclei); (c) presence and extent of BM fibrosis (as assessed according to the 2008 World Health Organization MF-scoring system) [1].

Immunohistochemical analysis

The architecture and immunohistochemical features of splenic stroma and red pulp vessels were assessed using antibodies against the following antigens: CD34 (clone QBEND/10; Leica Microsystems, Buffalo Grove, IL, USA), CD8 (clone C8/144B; Dako, Carpinteria, CA, USA), NGFR (clone ME20.4; Abcam, Cambridge, MA, USA), and SMA (clone 1A4; Dako). Antigen detection was performed with the Leica Refine Polymer Detection kit, using an automated BOND III immunostainer (Leica Biosystems). In MPN-MF cases, the number of SMA-positive and NGFR-positive stromal cells was assessed by comparison with control spleens and graded as increased, normal, or reduced. The density of CD8-positive splenic sinusoids and CD34-positive microvessels was esteemed by manually counting at least 10 representative high-power fields (HPF). For comparison between normal spleens, transplanted and nontransplanted MPN-MF cases, the mean values of CD34 and CD8 density were considered.

Results

Clinical–epidemiological features of the study population

To evaluate the splenic histologic response to HSCT in MPN-MF, the present study considered three subgroups of patients: (a) patients with a history of MPN-MF, treated with HSCT; (b) patients with history of MPN-MF, treated with conventional therapies other than HSCT; and (c) patients without hematological malignancies, who underwent splenectomy during major abdominal surgery. The clinicopathological features of each subgroup are reported in Table 1.

The post-HSCT MF subgroup included two men and one woman (mean age at splenectomy, 46.7 years; range, 33–55 years). Pretransplant Lille score, Dynamic International Prognostic Scoring System score, Eastern Cooperative Oncology Group performance status, number of circulating blasts, and lactic acid dehydrogenase levels are reported in Table 2. In all cases, HSCT was performed using peripheral mobilized blood stem cells from matched unrelated donors and reduced-intensity conditioning regimens (fludarabine *plus* melphalan: 2 cases; fludarabine *plus* busulfan: 1 case; Table 2) [20,21]. Complete histologic response in the BM was achieved in all cases at different time points after HSCT: two cases (#1 and #2) achieved complete histologic response <3 months after transplant; one case (#3) attained only partial response 1 year after HSCT, but subsequent follow-up showed complete clinical and histologic response (CR) [19]. Of note, the patient attained CR after splenectomy without any further treatment. As for the long-term outcome, two patients (Cases #1 and #2) died of infectious complications 3.7 months and 14.8 months after HSCT, before complete normalization of the peripheral blood counts; one patient (Case #3) is alive and in CR 29.5 months after HSCT.

Post-HSCT splenectomy was performed due to refractory thrombocythopenia (Cases #1 and #2) or refractory cytopenia with hemolysis (Case #3). At the time of splenectomy, the latter patient presented with atypical hemolytic uremic syndrome. The mean interval between HSCT and splenectomy was 102.7 days (56 days in Case #1; 76 days in Case #2; and 176 days in Case #3). At the time of splenectomy, all patients were under treatment with tacrolimus (Tables 1 and 2).

Table 1 Clinico-pathological features of the study population.

Patient	Group	Sex	Age (y)	Diagnosis	Post-HSCT (d)	Spleen weight (g)	Ganma—Gandy bodies	EMH score	EMH pattern	EMH lineage	Fibrosis	CD34+ vessel density[b]	CD8+ sinusoid density[b]	SMA+ & NGFR+ cells
1	Post-HSTC MF[a]	M	55	PMF	56	3100	Present	0	—[c]	—[c]	Present	42.1	19.5	Increased
2	Post-HSTC MF[a]	F	52	Post-PV PMF[d]	76	1540	Present	1+	Diffuse	Erythroid	Present	35.8	11	Increased
3	Post-HSTC MF[a]	M	33	PMF	176	2560	Present	2+	Nodular and diffuse	Mixed	Present	38.5	15.3	Increased
4	Nontransplanted MF	M	71	PMF[d]	NA	5400	Absent	3+	Nodular and diffuse	Mixed	Present	41.4	29.5	Increased
5	Nontransplanted MF	M	63	PMF	NA	1126	Rare	3+	Nodular and diffuse	Mixed	Present	39.9	10.2	Increased
6	Nontransplanted MF	F	72	Post-PV PMF[d]	NA	2933	Absent	3+	Nodular and diffuse	Mixed	Present	39.2	17.8	Increased
7	Normal control	M	76	LMS	NA	120	Absent	0	—[c]	—[c]	Absent	27.1	48.6	Normal pattern
8	Normal control	M	54	Esophageal carcinoma	NA	58	Absent	0	—[c]	—[c]	Absent	34.2	46.1	Normal pattern
9	Normal control	M	69	Pancreatic carcinoma	NA	150	Absent	0	—[c]	—[c]	Absent	32.7	41.2	Normal pattern

Note. EMH = extramedullary hematopoiesis; F = female; HSCT = hemopoietic stem cell transplant; LMS = leiomyosarcoma; M = male; NA = not applicable; PMF = primary myelofibrosis; PV = polycythemia vera.

[a] GVHD prophylaxis/other treatments at the time of splenectomy: tacrolimus (patients 1 and 2); tacrolimus *plus* methylprednisone (patient 3).

[b] Number of vessels/10 high power fields.

[c] No EMH identified.

[d] *JAK2*^{V617F}-positive case.

Table 2 Detailed clinical features and outcome of the three transplanted patients.

Patient #	Lille Score[a]	DIPPS Score[a]	ECOG Score[a]	Peripheral blasts (per 10^3 WBC)[a]	LDH (IU/L)[a]	Conditioning	CD34 dose ($\times 10^6$/kg)	Indication to splenectomy	Response to splenectomy	Outcome	Survival (mo)
1	Int	Int-2	1	16	1260	Flu/mel	10.5	Refractory thrombocytopenia	Normalization of platelet count	Dead of infection	14.8
2	High	High	2	8	288	Flu/mel	6.4	Refractory thrombocytopenia	Too short time to assess response.	Dead of infection	3.7
3	Int	Int-2	2	11	668	Flu/bu	4.7	Pancytopenia and hemolysis	Normalization of blood counts	Alive	29.5

Note. DIPSS = Dynamic International Prognostic Scoring System; ECOG = Eastern Cooperative Oncology Group; Flu/bu = fludarabine *plus* busulfan; Flu/mel = fludarabin *plus* melphalan; LDH = lactic acid dehydrogenase.

[a] Values before hemopoietic stem cell transplant.

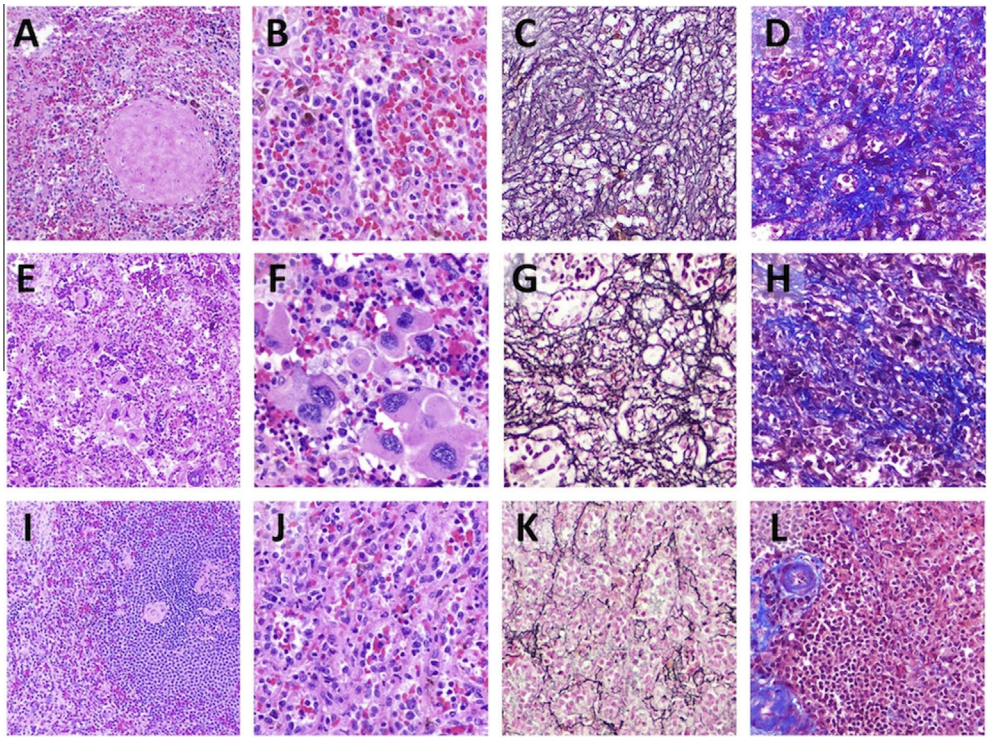

Fig. 1 Representative histologic features of the studied spleens. A-D. Post-HSCT MPN-MF spleens. The spleens from transplanted MF patients disclosed a marked reduction of EMH with abundant Gamna-Gandy bodies (A). Residual hemopoietic aggregates were small and mainly composed of erythroid precursors (B). Reticulin (C) and trichrome (D) stain disclosed marked splenic fibrosis with deposition of collagen fibers. E-H. Nontransplanted MPN-MF spleens. The spleens from nontransplanted patients were characterized by striking EMH (E), with large clusters of atypical megakaryocytes (F). However, with reticulin (G) and trichrome (H) stains, the degree of splenic fibrosis was similar to that oserved in transplanted MF. I-L. Normal controls. Normal spleens showed a normally preserved white pulp (I), with no evidence of EMH (J). Reticulin (K) and trichrome (L) stain showed absence of fibrosis. (H&E, reticulin and trichrome stains; original magnification 10x and 20x).

The nontransplanted MPN-MF subgroup included two men and one woman (mean age at splenectomy, 68.7 years; range, 63—72 years). These patients were treated with cytoreductive therapies, including cladribine *plus* ruxolitinib, interferon-α *plus* hydroxyurea, and hydroxyurea alone (1 case each). Splenectomy was performed for severe and untreatable cytopenia (2 cases) or massive splenomegaly (1 case). HSCT was not performed due to poor performance status, excessive comorbidities, and/or unavailability of a suitable graft. The normal spleen subgroup encompassed three men, with a mean age at splenectomy of 66.3 years (range, 54—76 years). These patients underwent splenectomy during major abdominal surgery for malignant tumors (leiomyosarcoma, esophageal carcinoma, and pancreatic carcinoma: 1 case each). Gross and histologic examination of the spleen excluded the presence of metastatic disease.

Spleen pathology in transplanted and nontransplanted MPN-MF

The spleens in transplanted and nontransplanted MPN-MF were markedly enlarged compared with normal controls (Table 1). The mean weight of post-HSCT spleens was lower than nontransplanted cases (mean spleen weight: 2400 g in post-HSCT MPN-MF; 3153 g in nontransplanted MPN-MF). Subcapsular infarcts were present in both transplanted and nontransplanted cases (post-HSCT MPN-MF: 2 cases; nontransplanted MPN-MF: 1 case). Splenic vessels at hilum were regular, with no evidence of thrombosis.

In comparison to normal controls, the spleens of transplanted and nontransplanted MF cases showed marked atrophy of the white pulp, with congested and effaced red pulp. Gamna—Gandy bodies were abundant in all post-HSCT cases, rare or absent in nontransplanted MPN-MF, and completely lacking in normal controls (Table 1 and Fig. 1).

Significant differences in the presence and composition of EMH were observed between transplanted and nontransplanted MF spleens. In post-HSCT, residual EMH was either absent (Case #1) or mild to moderate (EMH score 1+ in Case #2; EMH score 2+ in Case #3). In Case 2, residual EMH was almost exclusively composed by erythroid precursors, preferentially located within the splenic sinusoids. In Case 3, residual EMH was composed of erythroid and myeloid cells, with rare atypical megakaryocytes within the splenic cords and sinusoids. The EMH growth pattern was diffuse in the first case and mixed (nodular and diffuse) in the other. By contrast, in nontransplanted MPN-MF, splenic EMH was widespread and severe (score 3+), featuring aggregates of myeloid and erythroid precursors, with occasional clusters of atypical megakaryocytes. The growth pattern was mixed (nodular and diffuse) in all cases. EMH was located in both the splenic sinusoids and red pulp cords (Table 1 and Fig. 1).

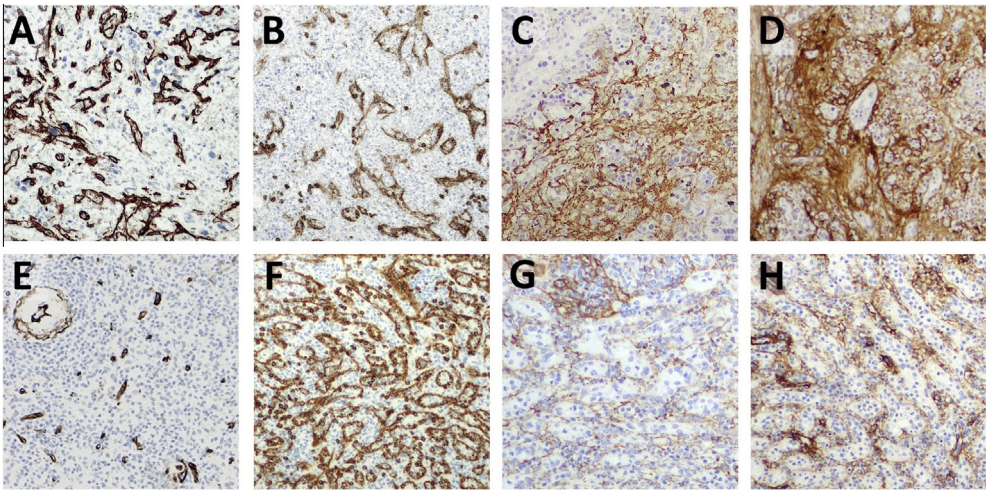

Fig. 2 Immunohistochemical features of the studied spleens. A-D. MPN-MF spleens. Both the transplanted and non-transplanted spleens in MPN-associated MF were characterized by a high density of CD34-positive microvessels (A), a decreased frequency of CD8-positive splenic sinusoids (B) and a high frequency of SMA-positive myoid cells (C) and NGFR-positive adventitial reticulum cells (D). The immunohistochemical features of post-HSCT spleens were comparable with those of non-transplanted cases (not shown). E-H. Normal controls. In normal spleens, the density of CD34-positive vessels (E) was significantly lower than in MF cases. Note the normal frequency of CD8-positive sinusoids (F). The SMA-positive myoid cells (G) and NGFR-positive adventitial reticulum cells (H) were normally distributed, found mostly around the splenic vascular structures. (Immunoperoxidase and reticulin stain; original magnification 10x and 20x).

In relation to splenic fibrosis, both transplanted and non-transplanted MF disclosed reticulin and collagen deposition. By contrast, normal controls did not show evidence of fibrosis. In MF cases, splenic fibrosis was not influenced by the *JAK2* mutational status and/or by the degree of EMH (increased reticulin and collagen fibers were documented even in the spleen without residual EMH after HSCT; Table 1 and Fig. 1).

No significant differences in spleen histology were noted between $JAK2^{V617F}$-positive and $JAK2^{V617F}$-negative MF patients in both the transplanted and nontransplanted subgroups. Also, the type and number of prior therapies did not correlate with any considered histological parameter.

Immunohistochemical analysis of the red pulp vessels and stromal cells disclosed differences between MF cases and normal controls. Both transplanted and nontransplanted MPN-MF spleens showed increased numbers of CD34-positive microvessels compared with normal spleens (mean CD34-positive microvessel density: 38.8/HPF in post-HSTC MF; 40.2/HPF in nontransplanted MF; 31.3/HPF in normal spleens). The increase in CD34-positive microvessels was associated with a reduction of CD8-positive sinusoids (mean sinusoid density: 15.3/HPF in post-HSCT MF; 19.2/HPF in nontransplanted MF; 45.6/HPF in normal spleens; Table 1 and Fig. 2). Compared with normal spleens, MPN-MF cases had more SMA-positive myoid cells and more numerous NGFR-positive adventitial reticulum cells. The quantity and distribution of SMA-positive and NGFR-positive cells roughly paralleled the extent and distribution of spleen fibrosis (Fig. 2).

No differences in CD34-positive microvessels, CD8-positive sinusoids, SMA-positive myoid cells, or NGFR-positive adventitial reticulum cells were observed between transplanted and nontransplanted MPN-MF. Of note, the number of SMA-positive or NGFR-positive cells did not correlate with the presence/extent of EMH.

Comparison between spleen and BM histology

Correlations between splenic and BM histology were also investigated. Post-HSCT BM biopsies at the time of splenectomy were available for two patients: one case with mild splenic EMH, who achieved complete BM histologic response <3 months after HSCT (Case #2); and one case with EMH score 2+ and delayed CR (Case #3). In the first case, the BM disclosed normal cellularity, with normal hematopoiesis, no atypical megakaryocytes, and MF1 fibrosis, consistent with CR [19]. In the second case, the BM biopsy showed increased cellularity for patient age (90%), with atypical megakaryocytes and MF1 fibrosis, consistent with clinical improvement (revised International Working Group-Myeloproliferative Neoplasms Research and Treatment and European LeukemiaNet criteria) [19].

Discussion

PMF and post-PV MF are hematological neoplasms, characterized by largely overlapping clinicopathologic features and by a poor prognosis [22—24]. In spite of its significant morbidity and mortality, HSCT remains the only curative therapeutic modality with high CR rates and significant improvement of the disease natural history [25,26]. Although the marrow histologic features of advanced stage PMF, post-PV, and post-ET MF are well described [1,4,27—30] and several studies have documented changes in pre-HSCT *versus* post-HSCT BM [31—34], very little is known on post-HSCT spleen histology in patients with MPN-MF. In

addition, no data are available on the relation between BM changes (i.e., decreased cellularity, presence of atypical megakaryocytes, degree of BM fibrosis) and any possible change in spleen histology. To address these issues, we compared the histologic features of spleens from transplanted and nontransplanted MPN-MF patients. Control spleens were also evaluated for comparison. Spleen histology in transplanted MF patients was compared with marrow findings at the time of splenectomy.

Our results demonstrate that the weight of post-HSCT MF spleens was markedly lower than that of nontransplanted cases (2400 g vs. 3153 g) and that this difference is mostly due to reduced EMH amounts in transplanted MF compared with nontransplanted cases (Table 1). In addition, the histologic composition of EMH significantly differed between transplanted and nontransplanted cases, as large clusters of atypical megakaryocytes were only faced in the nontransplanted group (Table 1 and Fig. 1). These differences constitute the first histologically documented evidence that HSCT reduces or abrogate neoplastic EMH in the spleen.

Gamna—Gandy bodies (sclero-siderotic nodules) were largely restricted to the transplanted group. The reasons of this finding are still unknown, but some explanatory mechanisms may be proposed. Gamna—Gandy bodies are the histologic hallmark of microvascular damage and scarring. In our series, they may thus represent the vascular cytotoxic effects of either conditioning or post-transplant immune suppressive therapy (i.e., tacrolimus). The second hypothesis may be more likely sustained by: (a) the well reported vascular toxicity of calcineurin-inhibitors [35]; (b) the relative long time interval elapsed between HSCT and splenectomy. Further studies are, however, needed to clarify this issue.

Irrespective of the etiology of vascular damage, it is possible to speculate that these injuries may affect the capability of the splenic vascular microenvironment in sustaining pathologic hematopoiesis. EMH cells indeed reside intravascularly in dilated splenic sinusoids or within abnormal hematopoietic niches, which include endothelial cells, fibroblasts, and histiocytes [36—39]. Endothelial cells and fibroblasts of MPN-MF can sustain the differentiation of neoplastic megakaryocytes [40—43], regulate the mobilization of neoplastic stem cells [38,44—46], and modulate the expression of several chemokines and adhesion molecules [40,41,47].

Compared with normal spleens, both transplanted and nontransplanted MPN-MF spleens were also characterized by a high density of CD34-positive microvessels, paralleled by a decreased density of CD8-positive sinusoids. SMA-positive and NGFR-positive stromal cells were both markedly increased in all MPN-MF cases, irrespective of prior treatments (Table 1 and Figs. 1 and 2). These changes are in keeping with what was reported in a large series of MPNs, including cases of PMF [16—18].

The absence of stromal changes after HSCT poses intriguing questions on the biological response to such therapy. Two hypotheses can indeed explain the persistence of stromal changes up to 176 days after HSCT: (a) less likely, an inefficacy of HSCT to induce a complete stromal normalization; and (b) more likely, the need of a long time for the improvement of stromal changes. Recent literature data seem to sustain the latter hypothesis. In particular, in MPN-MF, Ciurea and colleagues [33] reported a variable reduction of spleen size after HSCT, with subsets of patients requiring more than 36 months for complete resolution of splenomegaly. In such cases, delayed spleen responses may be attributable to a late resolution of red pulp fibrosis. Such hypothesis is also sustained by recent studies on BM histology in transplanted PMF patients, reporting a late reduction of interstitial fibrosis in a consistent subgroup of patients [31—34]. Similar time trends in fibrosis resolution may indeed characterize two organs (the spleen and the BM) involved by the same neoplastic process. This hypothesis is sustained by the roughly comparable histologic responses to HSCT found in our two patients with available matched spleen and BM samples. One patient (Case #2) showed complete disappearance of EMH in the spleen and normal cellularity in the BM, while the second (Case #3) showed residual neoplastic hematopoiesis in the spleen, paralleled by increased cellularity and atypical megakaryocytes in the BM.

In conclusion, our study demonstrates that HSCT induces marked histologic changes in the spleen of MPN-MF patients. The HSCT-mediated splenic effects parallel similar changes in the BM (normalization of cellularity and reduction of megakaryocyte atypia), suggesting a possible common mechanism in the response to therapy. In our series, HSCT did not significantly affect splenic fibrosis (more time may be needed to induce its regression). These results pose intriguing questions on MPN-MF biology and on the splenic effects of MF-related therapies. They are, however, inferred from a relatively small number of cases and need to be confirmed on larger and well-annotated cohorts of patients. This will help clarify the still open issues concerning the fascinating and complex topic of spleen involvement in MPN-MF.

Conflicts of interest

No financial conflicts to declare.

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