INTERNAL MEDICINE

Early Improvement in Marrow Fibrosis Following Haploidentical Stem Cell Transplantation for a Patient with Myelodysplastic Syndrome with Bone Marrow Fibrosis

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Abstract

The prognosis for myelodysplastic syndrome with bone marrow fibrosis (MDS-F) is worse than the prognosis of MDS without fibrosis. Hematopoietic stem cell transplantation (HSCT) is the only curative therapy; however, the indications and the procedures involved in HSCT remain unclear. We herein describe a 69-year-old Japanese man with MDS-F who received haploidentical HSCT and post-transplantation cyclophosphamide. Although the first HSCT resulted in secondary graft failure, the second HSCT using PTCy led to successful engraftment after early improvement in fibrosis. Since the incidence of graft failure is high in myelofibrosis patients, a secondary HSCT using PTCy may be successful if employed.

Key words: myelodyspastic syndrome, myelofibrosis, haploidentical hematopoietic stem cell transplantation, post-transplantation cyclophosphamide

Introduction

Bone marrow (BM) fibrosis develops in association with various conditions in addition to hematologic-neoplasms such as primary myelofibrosis (PMF), including autoimmune disease, vitamin D deficiency, and human immunodeficiency virus infection (1). Marrow fibrosis is thought to result from the activity of cytokines produced by the abnormal megakaryocytes associated with hematologic-neoplasms (2). Myelodysplastic syndrome (MDS) is a disorder associated with the development of myelofibrosis. Approximately 10-20% of patients with MDS have been reported to have BM fibrosis, and their outcomes were worse than the outcomes of patients without fibrosis (3, 4). HSCT is the only curative therapy for myelofibrosis including MDS with fibrosis (MDS-F), but the indications for using it and the procedures involved in HSCT remain unclear.

We report a patient with MDS-F who was successfully engrafted by second haploidentical (HAPLO-) HSCT that had been preceded by reduced intensity conditioning (RIC) and followed by posttranplantation cyclophosphamide (PTCy).

Case Report

A 69-year-old man was referred to our hospital because of pancytopenia. He presented with slight fatigue and pallor. The physical examination revealed petechiae on his legs but no other abnormal findings, including hepatomegaly and splenomegaly. His laboratory findings were as follows: while blood cell count 1.84×10^9/L (myeloblasts 1%, myelocytes 1%, segmented neutrophils 39%, basophils 2%, lymphocytes 57%), hemoglobin 10.3 g/dL, reticulocyte count 17.3×10^9/L, platelet count 14.0×10^9/L, lactate dehydrogenase 221 IU/L, total bilirubin 0.7 mg/dL, and serum creatinine
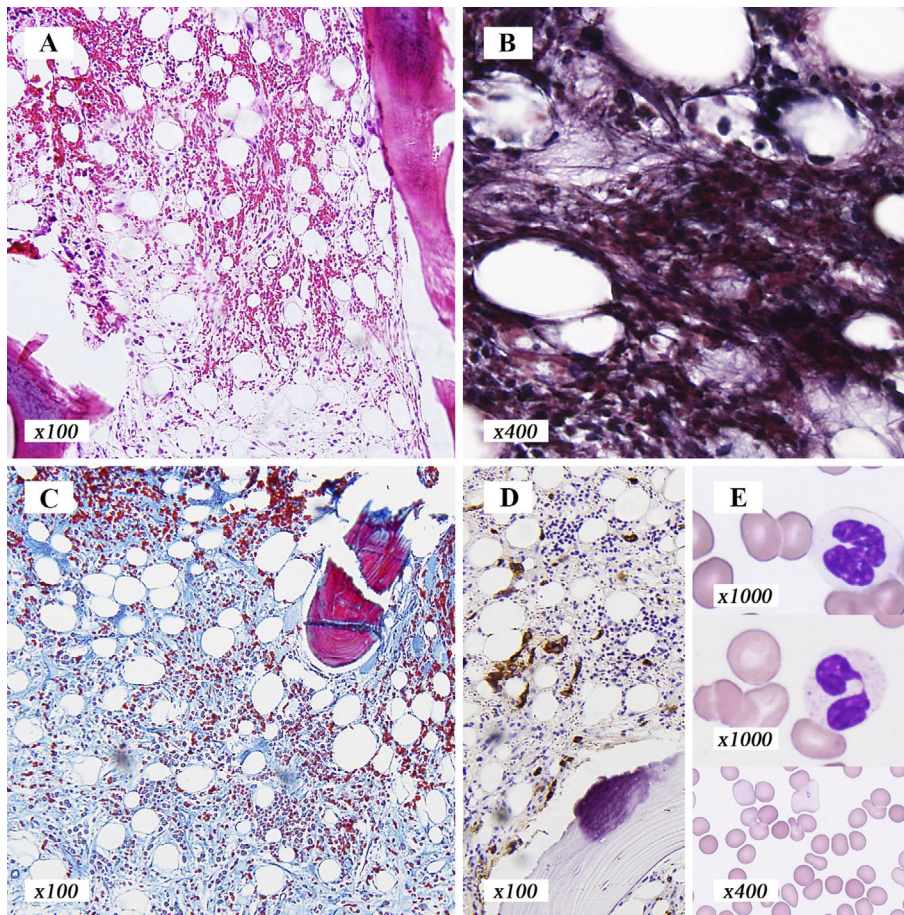
Figure 1. Bone marrow biopsy and peripheral blood smear at the time of diagnosis. The bone marrow is hypoplastic (A: Hematoxylin and Eosin staining). Diffuse reticulin (B: silver impregnation technique) and collagen fibrosis (C: Azan staining) with atypical megakaryocytes (D: Factor VIII immunohistochemistry staining) are seen. The marrow was classified as MF-2 by the European Consensus on grading bone marrow fibrosis. May-Giemsa staining of the peripheral blood smear shows agranular neutrophils (E top), pseudo Pelger-Huet anomaly (E middle), and erythrocyte anisocytosis (E bottom).

0.64 mg/dL. There were no nucleated red blood cells or tear-drop cells in the peripheral blood smear, but agranular neutrophils, pseudo Pelger-Huet anomaly, and erythrocyte anisocytosis were seen (Fig. 1E). A BM aspiration was unsuccessful, and the subsequent BM biopsy revealed hypoplasia and dysplastic megakaryocytes. There were diffuse fibrotic changes (Fig. 1A) mainly consisting of reticulin fibers that were positively stained by a silver impregnation technique (Fig. 1B). Azan staining and factor VIII immunohistochemistry staining showed a relative increase in the numbers of collagen fibers and megakaryocytes, respectively (Fig. 1C and D). The myelofibrosis grade was MF-2 according to the European consensus on grading BM fibrosis (5). The numbers of CD34-positive cells were not increased, but *p53* gene products were overexpressed in the BM (figures not shown). A chromosome analysis of the BM revealed 45,X,-Y in 5 and 46,XY,del(9)(q?) in 2 out of 20 metaphase cells. The *JAK2 V617F* mutation was not detected in the patient's peripheral blood. The presence of the myeloproliferative leukemia virus proto-oncogene (*MPL*) and the calreticulin mutation were not examined. Despite the presence

of marrow fibrosis, these hematologic findings did not satisfy the WHO criteria for primary myelofibrosis. Therefore, we diagnosed the patient with MDS-F based on the morphology of the peripheral blood smear, the chromosomal abnormalities, and the overexpressed *p53* gene products in the BM. The international prognostic score for MDS (IPSS) was intermediate-1, and the revised international prognostic score for MDS (IPSS-R) was intermediate (6).

Since the patient was 69 years old, and his pancytopenia was not severe when he was first seen, we initially decided to monitor his clinical course. Unfortunately, the pancytopenia progressed fairly rapidly, and 3 months after his diagnosis the patient presented to our emergency department with a high fever, disorientation, and facial palsy with right-sided hemiparesis. His laboratory data were as follows: white blood cell count 1.01×10^9/L (neutrophils 17.9%, basophils 1.0%, monocytes 15.8%, and lymphocytes 65.3% by an automated analyzer), hemoglobin 6.9 g/dL, reticulocyte count 85.1×10^9/L, platelet count 3×10^9/L, and C-reactive protein 6.7 mg/dL. Computed tomography (CT) revealed a left-sided subdural hematoma. No foci of infection were re-
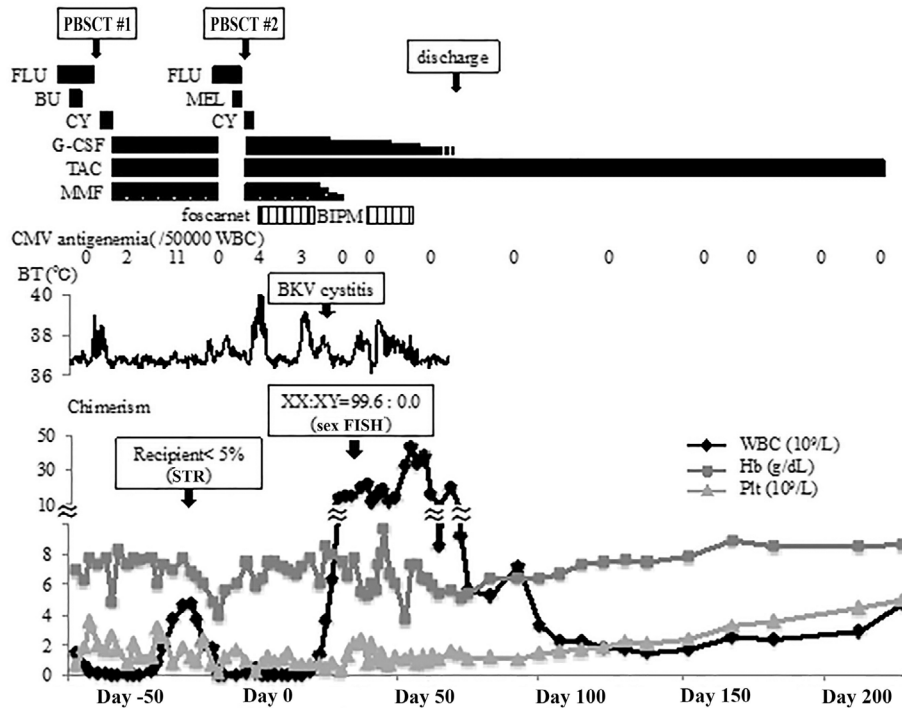
Figure 2. **Clinical course of hematopoietic stem cell transplantation (HSCT). The second HSCT was performed after graft failure of the first HSCT. BIPM: biapenem, BKV: BK virus, BT: body temperature, BU: busulfan, CMV: cytomegalovirus, CY: cyclophosphamide, FISH: fluorescent in situ hybridization, FLU: fludarabine, G-CSF: granulocyte colony stimulating factor, MEL: melphalan, MMF: mycophenolate mofetil, PBSCT: peripheral blood stem cell transplantation, Plt: platelet count, STR: short tandem repeats, TAC: tacrolimus**

vealed by CT; however, *Klebsiella pneumoniae* and *Streptococcus agalactiae* were isolated from a blood culture a few days after the patient was admitted.

The patient was admitted for evacuation of the hematoma and received antibiotics for febrile neutropenia. His disorientation and paresis resolved after surgery, but his pancytopenia worsened, and he required blood transfusions every few days and the daily administration of granulocyte-colony-stimulating factor (G-CSF). The IPSS-R category was changed from intermediate to high, and we decided to perform HSCT as soon as possible. A human leukocyte antigen (HLA)-matched sibling donor was not available, and there was not enough time to identify unrelated HLA-matched donors. Although there were some cord blood stem cell units available, the number of cells was not enough for the patient. The patient and his family chose HAPLO-HSCT because his condition was very serious and there was little time to prepare for treatment. Therefore, the patient underwent HAPLO-HSCT, with his son as the donor. The conditioning regimen consisted of 30 mg/m^2 fludarabine on Days -7 to -2 and 3.2 mg/kg intravenous busulfan on Days -5 to -4. The total number of infused CD34-positive cells was 3.14×10^6/kg. In addition, cyclophosphamide was administered at 50 mg/kg on Days 3 and 4. Tacrolimus and mycophenolate mofetil (MMF) were begun starting from Day 5 for immunosuppressive therapy. The patient developed a high fever associated with an allo-reactive response shortly

after the HSCT, but the fever improved after the administration of cyclophosphamide. On Day 20 of the first HSCT, the patient's neutrophil count was greater than 0.5×10^9 cells/L, and there was complete donor chimerism of the nucleated cells on Day 32 (recipient white blood cells <5% by a short tandem repeat analysis). However, his neutrophil count rapidly decreased from 4.55×10^9/L on Day 31 to less than 0.1×10^9/L on Day 38 of the first HSCT (Fig. 2). Because a BM biopsy (Day 35 of the first HSCT as Day -20 of the second HSCT in Fig. 3) revealed that the degree of marrow fibrosis had decreased, no hemophagocytosis had been seen, and the numbers of hematopoietic cells had slightly recovered, we thought that the findings indicated secondary graft failure. We decided to perform salvage HAPLO-peripheral blood stem cell transplantation with cells donated by the patient's daughter. On Day 49 of the first HSCT, we initiated a second conditioning regimen consisting of 25 mg/m^2 fludarabine on Days -6 to -2 and 40 mg/m^2 melphalan on Days -3 to -2. A total of 4.87×10^6/kg of CD34-positive cells were infused on Day 0 of the second HSCT, which was Day 55 of the first HSCT. The patient received the same immunosuppressive treatments as the first HSCT, consisting of PTCy, tacrolimus, and MMF. On Day 18 of the second HSCT, the patient's neutrophil count was greater than 0.5×10^9/L, and there was complete donor chimerism on Day 32 (donor white blood cells >99.6% by fluorescent *in situ* hybridization analysis). The neutrophil counts held steady de-
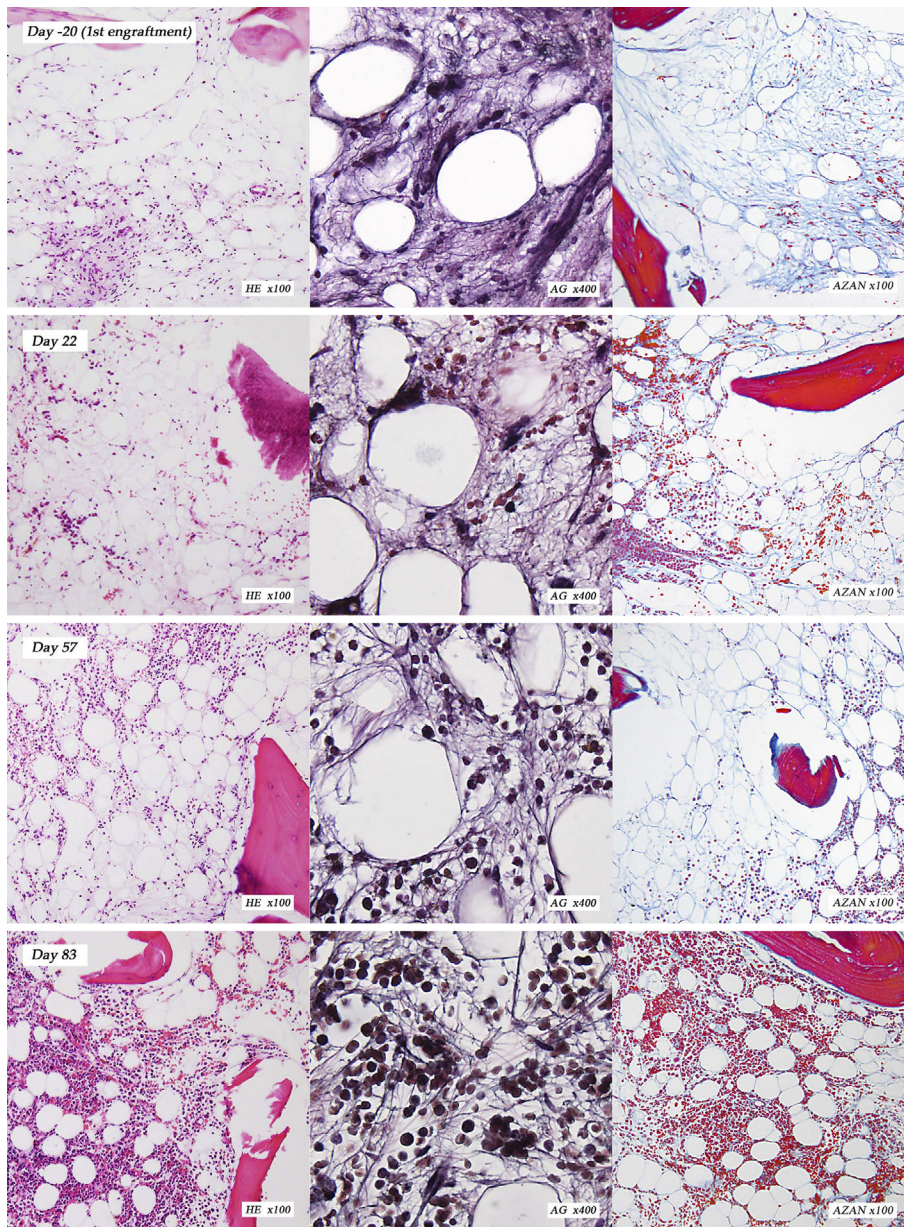
Figure 3. Change in degree of marrow fibrosis and hematopoietic cell recovery. At the first engraftment, the marrow fibrosis remained but was moderately decreased, especially the reticulin fibrosis. Both the collagen and reticular fibrosis decreased further on days 22, 57 and 83 of the second HSCT and hematopoietic cells gradually recovered. HE: Hematoxylin and Eosin staining, AG: silver impregnation technique, Azan: Azan staining

spite the tapering of G-CSF. The reticulocyte and platelet counts gradually increased. The patients developed several complications, including BK virus-associated hemorrhagic cystitis, cytomegalovirus reactivation, and antibiotics-related kidney damage, but none were serious. On Day 69 of the second HSCT, the patient was ambulatory and was discharged. Since no symptoms of graft-versus-host disease (GVHD) were observed, the administration of tacrolimus was stopped on Day 194 of the second HSCT. On Day 245, the patient had laboratory data and symptoms of chronic GVHD involvement of the liver and the mouth, which were less than SCORE 2 of the organ scoring system of chronic GVHD by the National Institutes of Health (7). The patient

was started on cyclosporine. The GVHD was not severe but prolonged, so the administration of cyclosporine was continued to Day 635 of the second HSCT.

He underwent BM biopsy on the right side of his posterior iliac crest every several weeks to check his hematopoietic status and to evaluate the BM fibrosis. At his first engraftment, the reticulin fibrosis was moderately decreased, while the collagen fibrosis was minimally decreased. After the second HSCT, both types of marrow fibers gradually disappeared, while there was gradual recovery of the hematopoietic cells on biopsies performed on Days 57 and 83 (Fig. 3). The patient was alive and in fairly good health 28 months after the HSCTs. BM biopsy on Day 601 (20

months) showed a fibrosis level of MF-0.

Discussion

Compared with MDS without fibrosis or with mild fibrosis, the cumulative incidence of non-leukemic death and leukemic evolution is much higher in MDS with moderate-to-severe fibrosis (8). Our patient's BM was severely hypoplastic and mostly consisted of reticular fibers and collagen fibers. Given the condition of his BM and the poor prognosis of MDS-F, we had no choice but to perform life-saving HSCT. However, graft failure occurred after the first HSCT. The main causes of graft failure have been found to be immune rejection mediated by residual cellular immunity or humoral immunity, defects in the host BM microenvironment, and viral infections (9).

Some studies have reported the impact of BM fibrosis on the outcomes of patients with MDS-F after HSCT. The European Group for Blood and Marrow Transplantation (EBMT) study (10) found that the cumulative incidence of engraftment at Day 30 was lower in patients with fibrosis than in patients without fibrosis. Severe fibrosis was the single negative predictive factor affecting the three-year disease-free survival and overall survival rate after HSCT. However, Scott et al. found that marrow fibrosis did not affect the outcome of HSCT for patients with early-stage MDS whose IPSS was low or intermediate-1; however, marrow fibrosis had negative impact on the outcome of patients with advanced disease whose IPSS was intermediate-2 or high (11). Our patient was considered to have IPSS intermediate-1 risk or IPSS-R high-risk disease, and his degree of fibrosis was moderate to severe (MF-2 by the European consensus on grading BM fibrosis). According to these reports, our patient had some risk of graft failure. The data from the Center for International Blood and Marrow Research on the outcome of transplantation for myelofibrosis show that engraftment occurred in 95%, 73%, and 83% of matched sibling donors, alternative related donors, and unrelated donors, respectively (12). The graft failure rate of HSCT for myelofibrosis compared with HSCT for other hematological malignancies was not very high for related sibling donors but was higher for alternative related donors and unrelated donors. After the abnormal megakaryocytes were eliminated by the conditioning regimen of the first HSCT, the degree of marrow fibrosis seemed to decrease, leading to the expectation of eventual HSC engraftment. We did not check the HLA antibody status of our patient. He did not develop viral infections during the first HSCT. There were no mismatches in the ABO blood groups. Taken together, the BM findings and outcome of the first HSCT, including the achievement of complete donor chimerism just 6 days before the graft failure, suggest that the cause the graft failure was poor graft function, possibly due to a poor host microenvironment given the fibrosis, and not immune-mediated rejection.

After Day 22 of the second HSCT, our patient's residual marrow fibrosis decreased further, and the peripheral blood cell counts gradually increased. In addition to the elimination of the abnormal megakaryocytes by the conditioning regimen, the graft verses leukemia effect might have been involved in the marrow improvements in fibrosis. There have been reports of the resolution of both collagen and reticulin fibrosis after successful stem cell transplantation for chronic idiopathic myelofibrosis (13-16). Although the marrow biopsies in these reports were performed at three months or later post-HSCT, the regression of fibrosis had already been recognized on Day 35 of the first HSCT in our patient. The fibrosis might begin to regress in earlier phase of the post-HSCT than expected. To the best of our knowledge, there have not been any reports showing photomicrographic evidence of sequential changes in the marrow fibrosis and the recovery of hematopoietic cells after HSCT for patients with myelofibrosis (Fig. 1, 3).

We used an RIC regimen because of our patient's age (69 years). HSCT for myelofibrosis was performed using myeloablative conditioning (MAC) until around 2000; however, the outcomes were not very good, although the treatment was limited to relatively young patients. Because of the advanced age of most patients with myelofibrosis, HSCT with RIC appears to be more tolerable than HSCT with MAC (16, 17). Using the RIC regimen with PTCy, we were able to perform the second HSCT relatively soon (55 days) from the first HSCT, and the engraftment was achieved on Day 18 of the second HSCT.

Several studies have used a one-day nonmyeloablative regimen or fludarabine and alemtuzumab regimen for transplantation after graft failure. Salvage HAPLO-HSCT using an RIC without subsequent PTCy was reported by Yoshihara et al. to provide encouraging results (9). They used a conditioning regimen containing fludarabine, antithymocyte globulin, and low-dose total body irradiation, each of which seems to be an intensification of the immunosuppression regimen. Because the procedure was performed immediately following the graft failure, immune-mediated rejection was thought to be one of the causes of the graft failure. We therefore used melphalan for the second HSCT, which is considered to be a drug that intensifies immunosuppression. In contrast, PTCy was thought to induce immune tolerance, which mitigates both graft rejection and GVHD after a HSCT with major histocompatibility complex mismatch (18). In our patient, no severe GVHD was observed throughout the entire course.

If no HLA-identical donors are available, umbilical cord blood or HLA-mismatched donor transplantation can be an option for HSCT for myelofibrosis. A multicenter study using alternative donor transplantation after RIC for individuals with advanced or high-risk leukemia or lymphoma compared the outcomes between those receiving double cord blood grafts and those receiving related haploidentical BM grafts with PTCy. The study confirmed the utilities of both types of alternative grafts for transplantation (18). Takagi et al. reported that umbilical cord blood transplantation follow-

ing RIC may be feasible, even for patients with severe marrow fibrosis (19). However, the overall survival in their study was poor. The efficacy and safety of HAPLO-HSCT is still unclear. HAPLO-HSCT with PTCy is a novel strategy that has provided very good outcomes in the field of hematological malignancy (20). Recently, Brodsky et al. described patients who underwent RIC followed by HAPLO-HSCT with PTCy for paroxysmal nocturnal hemoglobinemia, suggesting that HAPLO-HSCT might be a promising approach for patients with life-threatening nonmalignant or nonaggressive malignant hematologic disease who lack an HLA-matched donor (20). There have not been any reports on the use of HAPLO-HSCT with PTCy for myelofibrosis. To the best of our knowledge, this is the first case report to describe the use of HAPLO-HSCT with PTCy for a patient with MDS-F.

Conclusion

Reduced-intensity conditioning followed by HAPLO-HSCT with PTCy may be a promising therapeutic strategy for patients with MDS-F, and may even be safe for elderly patients. The early disappearance of reticulin-fiber-rich fibrosis was observed in the BM of our patient after the first HSCT. The elimination of abnormal megakaryocytes by the conditioning chemotherapy can be considered one of the causes of the improvement. If graft failure occurs, a second HAPLO-HSCT using RIC and PTCy can be considered as an option and it should be performed as soon as possible, since the marrow condition will have improved over the condition before the first attempt.

The authors state that they have no Conflict of Interest (COI).

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