Abstract

Myeloid metaplastia with myelofibrosis (MMM) is a chronic myeloproliferative disorder (CMPD) characterized by progressive anemia, massive splenomegaly, both hepatosplenic and non-hepatosplenic extramedullary hematopoiesis (EMH), a leukoerythroblastic blood smear, circulating progenitor cells, and marked bone marrow stromal reaction including collagen fibrosis, osteosclerosis and angiogenesis. The overall median survival is 5 years although it might range from 2 to 15 years depending on the presence or absence of clinically defined prognostic factors. Death is often due to leukemic transformation, portal hypertension or infection. In addition to shortened survival, quality of life is often affected by frequent red blood cell transfusions, profound constitutional symptoms, and cachexia. Drug therapy and autologous hematopoietic stem cell transplantation (HSCT) are of only palliative value and have not been shown to improve survival. The role of allogeneic HSCT, both myeloablative and non-myeloablative, is actively being investigated. Both splenectomy and radiation therapy have defined therapeutic roles to control EMH-associated symptoms. Analysis of the molecular biology of the disease is underway with the aid of animal models leading to the identification of novel therapeutic targets. Among the novel agents tested, thalidomide seems the most promising although newer agents are on the horizon. (Internal Medicine 43: 540–547, 2004)

Key words: myelofibrosis, myeloid metaplasia, myeloproliferative disorder

Introduction

Myelofibrosis with myeloid metaplasia (MMM), also known as agnogenic myeloid metaplasia (AMM) or idiopathic myelofibrosis, is a clonal hemopoietic stem cell disorder that is clinically characterized by progressive anemia, marked splenomegaly, extramedullary hematopoiesis, constitutional symptoms and a significant risk of evolution into an acute leukemia (1, 2). The disease is classified as a chronic myeloproliferative disorder together with polycythemia vera (PV) (3) and essential thrombocythemia (ET) (4). Characteristic laboratory features include teardrop poikilocytes and a leukoerythroblastic picture as well as circulating progenitor cells, all being a manifestation of bone marrow fibrosis and osteosclerosis. In general, MMM is a disease that affects the older population with the median age at diagnosis being 60 years. However, in a small number of patients (10%), the disease is diagnosed before age 40 years (5). MMM has also been described in children where it seems to have a more benign course (6). Disease incidence is estimated from 0.5 to 1.5 per 100,000 population per year although the incidence may be higher in Ashkenazi Jews (7–9).

Pathogenesis

MMM is a true stem cell disorder

Studies based on X-chromosome inactivation, cytogenetic lesions as well as ras gene mutation analysis, provide ample support for the stem cell origin of this disorder (10–12). It appears that the disease process affects a very primitive hemopoietic stem cell that can differentiate along both myeloid as well as lymphoid lineages (12). Morphologically, the bone marrow demonstrates an expanded population of dysplastic megakaryocytes, sinusoidal dilatation and intra-vascular hematopoiesis (Fig. 1). This is accompanied by the deposition of excessive amounts of collagen (types I and III) and other extracellular matrix proteins such as fibronectin and proteoglycans together with the formation of new bone (osteosclerosis), and blood vessels (angiogenesis) (13). These extracellular proteins are synthesized by polyclonal fibroblasts, stromal and smooth muscle cells in response to the cytokine milieu to which they are exposed (see below) (14). At the present time, the source(s) of these fibrogenic cytokines are not clear and both malignant megakaryocytes

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as well as cells of the monocyte-macrophage lineage have been implicated (15, 16).

**Megakaryocytes, thrombopoietin, and MMM**

The profound increase of abnormal clonal megakaryocytes seen in this disorder has stimulated considerable interest regarding their role in the etiology of this disease. Thrombopoietin is the main regulator of megakaryocyte and platelet homeostasis (17). Patients with MMM have elevated levels of TPO in the circulation (18, 19). While megakaryocytes from patients with MMM can grow in the absence of TPO, they are still responsive to the effect of the cytokine (20). These observations prompted the development of models of the disease whereby laboratory animals are exposed to chronically elevated levels of TPO either administered exogenously or continuously by gene transfer using vectors (21–24). These mice develop a bone marrow failure syndrome similar to human MMM and die within a few months due to progressive disease. However, TPO alone cannot lead to the profound stromal reaction that is so typical of this disease.

**Fibrogenic, angiogenic, and osteogenic cytokines in MMM**

Over the last few years an increasing number of cytokines have been implicated in the etiopathogenesis of this intense stromal reaction. These include transforming growth factor beta 1 (TGF-β1), basic fibroblast growth factor (bFGF), platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), osteocalcin and osteoprotegerin (OPG). Some of these cytokines (TGF-β1, PDGF) are stored in megakaryocyte alpha granules.

Recent data suggests that patients with MMM have abnormal and elevated neutrophil or eosinophil emperipolesis through megakaryocytes. These megakaryocytes express abnormal levels (quantity and location) of P-selectin, an important mediator of neutrophil rolling. Neutrophils present in the cytoplasm of megakaryocytes exhibit signs of activation with release of their proteolytic enzymes leading to destruction of both cell types, potentially causing the release of TGF-β1 and PDGF from the alpha granules (25). This enhanced emperipolesis may also explain the increased release of neutrophil elastase and active MMP-9 present in patients with MMM that may be responsible for the increased levels of circulating CD34+ progenitor cells (26).

While PDGF stimulates the proliferation of fibroblasts, mesenchymal and smooth muscle cells, TGF-β1 stimulates these cells to synthesize and release extracellular matrix components such as collagen and proteoglycans. TGF-β1 release is temporally related to TPO stimulation and marrow
fibrosis does not occur in TPO stimulated mice if they do not express TGF-β1 (22, 27). Osteoprotegerin is an important inhibitor of osteoclastogenesis and animals engineered to over-express OPG develop osteopetrosis (28). Thrombopoietin up-regulates OPG expression in animal models and therefore can explain the osteosclerosis seen in this disease (29). MMM is associated with significant new vessel formation (13). Thrombopoietin also stimulates the release of VEGF from progenitor cells and stimulates them to develop along the endothelial lineage (30).

**GATA-1 and MMM**

While the above observations seem to form a cohesive story, other data suggests that megakaryocytes from patients with MMM can grow in the absence of TPO without having constitutive activation of its receptor (c-mpl) implying that other potential mechanisms may be responsible for the etiology of this disease (20). GATA-1 is a member of a recently identified group of zinc finger containing transcription factors that plays a prominent role in erythroid and megakaryocyte development (31, 32). While mice without any GATA-1 expression die in utero due to severe anemia, low level expression of the transcription factor is compatible with survival (31, 33). In the absence of GATA-1, megakaryocytes accumulate in the bone marrow and develop nuclear and cytoplasmic abnormalities and often fail to undergo endomitosis (34). These GATA-1−/− mice develop progressive anemia, marrow fibrosis, extramedullary hematopoiesis and have increasing numbers of circulating progenitor cells as they age (35). In addition, these mice have elevated levels of TGF-β1, PDGF, VEGF and osteocalcin in their bone marrow when compared to age-matched normal mice (35).

The potential role of GATA-1 in hemopoietic malignancies is further supported by the observation that acquired mutations in GATA-1 have been associated with transient myeloid disorder in patients with Down’s syndrome (36). However, the status of GATA-1 expression by hemopoietic cells in patients with MMM has been controversial. One group has reported no changes in GATA-1 expression in CD34+ cells isolated from patients with myelofibrosis (37). In contrast, another group has just reported in abstract form that GATA-1 levels are lower in progenitor cells isolated from patients with myelofibrosis (37). The two groups also report conflicting results in relation to friend of GATA-1 (FOG-1), an essential co-factor for GATA-1 function as a transcription factor. At present it is not clear how to reconcile these conflicting reports, although the methodology used was quite similar.

These two models are not necessarily implying different mechanisms but probably have a lot of common ground and both seem to recapitulate salient features of the human illness. While the association between TPO and GATA-1 expression is at present not fully understood, megakaryocytes from GATA-1−/− mice express low levels of c-mpl. TPO levels are not controlled at the level of transcription but by the rate of its clearance from the circulation (39). C-mpl present both on megakaryocytes as well as platelets, is responsible for TPO clearance from the circulation. Thus, the low levels of c-mpl expression, possibly due to low level expression of GATA-1 may in part explain the persistently high levels of TPO in these patients and therefore marrow fibrosis. If this model is correct, it further highlights the central role of the megakaryocytes in this disease both as an initiator (GATA-1 mutation) and perpetrator (TPO induced release of alpha granules, mitosis etc) (Fig. 2).

**Differential Diagnosis**

MMM is often suspected by the presence of leukoerythroblastosis in the peripheral blood smear (nucleated red blood cells, granulocyte precursors, teardrop-shaped erythrocytes), implying a bone marrow infiltrative process. A bone marrow examination is essential to differentiate between the different causes of both myelophthisis and bone marrow fibrosis. In MMM, leukoerythroblastosis is associated with bone marrow megakaryocytic hyperplasia, collagen fibrosis, osteosclerosis, and intramedullary sinusoidal hematopoiesis. The differential diagnosis includes other myeloid disorders (chronic myeloid leukemia, MDS with myelofibrosis, acute myelofibrosis), lymphoid neoplasms such as lymphoma and hairy cell leukemia, metastatic carcinoma as well as a variety of metabolic and inherited conditions such as hyperparathyroidism, lupus and others (40).

The majority of patients with MMM have chromosomal abnormalities at the time of diagnosis. These include simple deletions (13q and 20q), trisomy 8 or 9 and abnormalities on chromosome 1, 7 and 20 (41–44). Some of these abnormalities are relatively specific for MMM (1q and 13q) while others such as 5q are rare and therefore help in the diagnostic process (40).

**Treatment**

At the present time, the only curative modality for patients with MMM is allogeneic bone marrow transplantation (45). However, most patients with the disease are not eligible due to their age (more than 60 years old) and attendant co-morbidities. Thus, the aim of any therapeutic intervention must be designed to improve the quality of life and control symptoms related to the disease.

**Prognostic factors**

The overall median survival in MMM is approximately 5 years but death is not always due to MMM. Thus it is important to try and determine prognosis in patients with the disease at the time of diagnosis. A number of studies have shown that anemia (hemoglobin <10 g/dl), advanced age (>64 years), hypercatabolic symptoms (weight loss, profound fatigue, night sweats, low-grade fever), leukocytosis (>30,000/µl) or leukopenia (<4,000/µl), the presence of circulating blasts (≥1%), as well as various cytogenetic abnormalities (+8, 12p–) are independent adverse prognostic
factors and can be used for risk stratification (Fig. 3) (44, 46, 47). Patients with low risk disease can have an average life expectancy of more than 10 years. On the other hand, high-risk patients might survive less than 3 years from diagnosis. Not all patients with MMM require therapy and since none of the interventions currently in use has been shown to prolong survival, caution must be exercised when deciding who and when to treat so that the treatment does not get worse than the disease itself (48).

Therapeutic options

Patients with MMM may require therapy for a number of symptoms related to the disease including anemia, splenomegaly, elevated white cell or platelet counts, extramedullary hemopoiesis (EMH), pulmonary hypertension (PH), hypercatabolic symptoms and leukemic transformation. Therapeutic approaches to these manifestations of the disease are discussed in turn.

Treatment for anemia

Symptomatic anemia is the commonest indication for therapy in MMM. It is underscored that other causes of anemia are addressed before planning specific therapy for MMM-associated anemia (49). Early reports suggested that androgens may be of therapeutic benefit (50). Thus it is often recommended to treat the anemia with a combination of androgen such as fluoxymesterone (halotestin) 10 mg twice a day with prednisone 0.5 mg/day. With this regimen, up to 40% of patients may respond although responses are less likely if patients have massive splenomegaly or karyotypic abnormalities (51). If there is evidence of ongoing hemolysis, glucocorticosteroids alone may be sufficient to elicit a response. The combination of danazol (600–800 mg/day) and erythropoietin (EPO, 40,000 units/week subcutaneous injections) is also effective in controlling anemia in a

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**Figure 2.** Current understanding of the etiology of myeloid metaplasia with marrow fibrosis. A detailed explanation is presented in the text.

**Figure 3.** The Lille scoring system and prognosis in patients with myeloid metaplasia with myelofibrosis.

<table>
<thead>
<tr>
<th>Factor #</th>
<th>Risk Group</th>
<th>Median survival (mo)</th>
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<tbody>
<tr>
<td>0</td>
<td>Low</td>
<td>93</td>
</tr>
<tr>
<td>1</td>
<td>Intermediate</td>
<td>26</td>
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<td>2</td>
<td>High</td>
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subgroup of patients with MMM (52, 53). Sometimes EPO by itself may be able to improve the anemia in these patients (54).

Due to its pleotropic effects on cytokine production and its inhibitory effects on angiogenesis, thalidomide has received considerable attention as a therapeutic agent for MMM (55) as has been the case with multiple myeloma (56, 57). A dose of 200 mg per day leads to significant improvements in anemia and reduction of splenomegaly in a substantial proportion of patients (58). Patients seem to tolerate low doses of the drug well although 20% of them experience a myeloproliferative response characterized by leukocytosis and thrombocytosis (55). The combination of thalidomide (50 mg/day) and prednisolone started at 0.5 mg/kg per day and slowly tapered over the course of three months is associated with a higher response rate (anemia improved in 62% of patients treated) and lower toxicity (59). Despite these therapeutic interventions, many patients will ultimately become transfusion dependent as the disease progresses and they become refractory to the above-mentioned therapies.

Treatment for splenomegaly

Symptomatic splenomegaly is very common and a cause of significant morbidity in these patients. An enlarged spleen can cause pain, early satiety, diarrhea, worsening anemia and thrombocytopenia, portal hypertension and hypercatabolic symptoms. The drug of choice to control splenomegaly is hydroxyurea since it is well tolerated and dose adjustments can be easily performed to correct for responses (60, 61). If therapy with hydroxyurea fails, other drugs such as busulfan, melphalan and 2-chlorodeoxyadenosine may also be used for this purpose (62–65). While there have been reports that alfa interferon may control splenomegaly, in general it is poorly tolerated by patients with this disease (66–68). Therapy with these cytoreductive agents often leads to worsening cytopenias. Therefore, these agents are often combined with EPO in an attempt to limit drug-induced anemia and there is some evidence that such combinations may enhance the therapeutic effect of the regimen (54).

Patients with symptomatic splenomegaly that does not respond or cannot be treated with chemotherapy should be considered for splenectomy. The patients must be chosen carefully: ongoing disseminated intravascular coagulation must be controlled and other co-morbidities optimized. When splenectomy is performed by experienced surgeons, operative mortality is low (9% in the largest series reported) (69). Patients can expect to benefit from this procedure with decreasing transfusion requirements and improvement of hypercatabolic symptoms; but in general, thrombocytopenia does not respond. A significant fraction of patients will develop hepatomegaly due to extramedullary hemopoiesis and a little less than 25% will develop thrombocytopenia (69). The long term sequela of splenectomy in this group of patients is unclear at present. There has been suggestions that splenectomy may increase the risk of leukemic transformation and development of pulmonary hypertension (PH) (70, 71). However these may simply be manifestations of the natural history of the disease. Patients that are not suitable candidates for splenectomy may be treated with external beam radiation therapy to the spleen (100–500 cGy in 5–10 fractions). Radiation therapy may be associated with prolonged and significant cytopenias and responses are not typically long lasting (72).

Treatment for extramedullary hemopoiesis

Non-hepatosplenic extramedullary hemopoiesis (EMH) should be suspected in patients with unexplained paraspinal masses, spinal cord compression, pleural or pericardial effusions or ascites although these deposits have been anecdotally reported in virtually every organ system of the body (2). In this setting, technetium sulfur colloid imaging can assist in the diagnosis (73). Low-dose irradiation is very effective for EMH. Paraspinal/epidural masses can be treated with 1000 cGy in 5–10 fractions while pleural and peritoneal effusions can be resolved with 100–500 cGy in 5–10 fractions (2, 74, 75).

A small fraction of patients with MMM develop PH that can be quite symptomatic (71). The etiology of this is as yet unclear and probably multifactorial. Potential mechanisms include EMH as well as cytokine-induced pulmonary artery hyperplasia. Several groups have reported that serotonin may play an important role in the smooth muscle hyperplasia that is seen in patients with PH (76, 77). Platelets and megakaryocytes contain serotonin in their granules and its release in the pulmonary circulation might contribute to the origin of this serious complication in these patients. Pulmonary hypertension resulting from diffuse pulmonary EMH responds to whole lung irradiation with a single dose of 100 cGy (78).

Treatment for constitutional symptoms

Patients who develop severe hypercatabolic symptoms such as fever, night sweats, weight loss and profound fatigue should be screened for underlying infections. If symptoms are attributed to MMM, a therapeutic trial with etanercept, a tumor necrosis factor alfa (TNF-α) antagonist is reasonable (79). In an open label study, etanercept given at a dose of 25 mg subcutaneously twice weekly lead to significant symptomatic improvement in 60% of patients with some improvement in the anemia and splenomegaly in a smaller fraction of those treated. Therapy with this agent was well tolerated (79).

Leukemic transformation

A significant fraction of patients with MMM progress to overt acute myeloid leukemia (AML) (80). The median time to diagnosis was 31 months after recognition of MMM in one series. Despite therapy, patients who progress to AML have a very grim prognosis with a median survival of 2.6 months from diagnosis and aggressive therapy does not seem to have a major impact on survival in this group of patients (80).
Anemia
- Erythropoietin
- Fluoxymesterone
- Prednisone (Pred)
- Thalidomide±Pred
- Splenectomy
- Red cell transfusion

Splenomegaly
- Hydroxyurea
- Busulfan
- Melphalan
- Thalidomide
- Surgery
- Radiation

Hypercatabolic symptoms
- Hydroxyurea
- Thalidomide
- Etanercept
- Splenectomy
- Zarnestra (?)

EMH
- Radiation

Figure 4. Therapeutic options for patients with MMM.

Hematopoietic stem cell transplantation

In principle, allogeneic hematopoietic stem cell transplantation (HSCT) can cure patients with MMM. While a number of reports suggest that this is feasible and that successful engraftment occurs in the vast majority of patients, there are only anecdotal reports that cures have been achieved (81). Recently Deeg et al (82) reported their experience with allogeneic HSCT in patients with MMM. Their observations reinforce the fact that this procedure can be performed but it appears that patients with the least amount of fibrosis benefit the most. However, these patients are expected to have a better prognosis due to less advanced disease (82). Patients who undergo splenectomy before HSCT seem to engraft faster (83). Age has been reported as a major determinant of transplant outcome: patients younger that 45 years have a 5-year survival rate of 62%, otherwise only 14% survive 5-years post transplant (84). However, in another study, older patients had a 5-year survival rate of 50% (85). Transplant related mortality (TRM) is around 30% with a similar number of patients developing chronic and often extensive graft versus host disease (86). This high TRM has led investigators to evaluate the role of reduced intensity allogeneic HSCT in MMM (87). In the absence of leukemic transformation, TRM is further decreased with the use of autologous HSCT with significant improvements in anemia and splenomegaly observed in the majority of patients (88). Allogeneic HSCT (related or unrelated) may be a therapeutic option for young, high-risk patients who understand the risks involved. For the older patients with high-risk disease, reduced-intensity allogeneic and autologous HSCT might be reasonable options. However, good-risk patients should not be offered transplantation since in this group, the risks of transplant far outweigh the potential long-term benefits of the procedure.

Novel treatment options

Although PDGF is an important cytokine in the etiology of MMM, and its receptor is inhibited by imatinib mesylate, this agent has virtually no activity against the disease and is very poorly tolerated (89, 90). The farnesyl transferase inhibitor R115777 (Zarnestra) seems to have significant activity and controls symptomatic organomegaly and hypercatabolic symptoms (91). Preliminary data also suggests that an oral VEGF receptor inhibitor (PTK787/ZK 222584) may also have a role in controlling symptomatic splenomegaly and stabilize the disease (92). We are currently testing the therapeutic value of combination therapy consisting of thalidomide and prednisone with either etanercept or cyclophosphamide. We are also planning to open a treatment protocol using the thalidomide analog, revimid, that has been shown to be more potent than the parent drug.

Conclusion

While MMM remains an incurable illness, the last few years have witnessed considerable progress in the understanding of the etiology of this disease. An increasing number of molecular targets for potential therapy have been identified. Coupled with the availability of ever more realistic animal models, cure for this disease has never been closer.

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