Circulating hematopoietic progenitor cells predict survival in patients with myelofibrosis with myeloid metaplasia

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Background and Objectives. The levels of circulating hematopoietic progenitor cells are often increased in myelofibrosis with myeloid metaplasia (MMM). The prognostic relevance of this phenomenon is largely unknown.

Design and Methods. We determined the number of circulating myeloid (CFU-GM), erythroid (BFU-E), and pluripotent (CFU-GEMM) progenitors, in 110 patients with MMM at diagnosis using a semi-solid colony assay. Overall survival was investigated by plots of the Kaplan-Meier estimator; known risk factors and the number of circulating progenitor cells were tested by univariate and multiple Cox regression analysis.

Results. Univariate analysis showed that hemoglobin concentration (p=0.019), CFU-GM (p< 0.0001), BFU-E (p=0.002), and age (p=0.002) predicted survival. Numbers of circulating CFU-GM above the 75th percentile were associated with a significantly shorter survival than were CFU-GM levels at or below the 75th percentile (27 vs. 74 months, p=0.0007). Similarly, high numbers of BFU-E in peripheral blood (> 75th percentile) predicted a shorter survival (33 vs. 74 months; p=0.007). When myeloid and erythroid progenitor cells were calculated separately in the multiple Cox regression analysis, both CFU-GM (hazard ratio 2.8, 95% CI, 1.35 to 5.93) and BFU-E (hazard ratio 2.57, 95% CI, 1.26 to 5.21) numbers above the 75th percentile were independent adverse prognostic factors in our patients.

Interpretation and Conclusions. High levels of circulating myeloid and erythroid progenitor cells are novel risks factors in patients with MMM. The assessment of hematopoietic progenitor cells may be useful to determine risk-adjusted treatment strategies.

Key words: myelofibrosis with myeloid metaplasia, circulating hematopoietic progenitor cells, survival.

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Myelofibrosis with myeloid metaplasia (MMM) is a chronic myeloproliferative disorder (CMD) characterized by varying degrees of bone marrow fibrosis and extramedullary hematopoiesis. Characteristic morphologic findings in peripheral blood include teardrop-shaped and nucleated red cells, immature neutrophils, and abnormally large platelets. Anemia and elevated leukocyte and platelet counts are common findings at diagnosis, but thrombocytopenia often develops with disease progression.[1-7]

Already 30 years ago, Paul Chervenick demonstrated an increased number of myeloid progenitor cells (colony forming units-granulocyte/macrophage, CFU-GM) in the peripheral blood of patients with MMM.[8] These findings have been extended by using semi-solid colony assays and it is now generally accepted that erythroid progenitor cells (burst forming unit-erythroid, BFU-E) and pluripotent progenitors (CFU-GEMM) are also elevated in MMM.[9,10] Increased numbers of colony-forming cells can also be found in blood of patients with other forms of CMD, although there are typically fewers precursor cells in patients with polycythemia vera and essential thrombocythemia than in patients with MMM.[10-12] In agreement with the concept that the circulating pool of precursor cells is increased in CMD, higher numbers of CD34$^+$ cells have been demonstrated by flow cytometry.[13,14]

MMM cannot be cured by conventional therapies. No therapy has yet been shown to offer a survival benefit or to slow the progression of bone marrow fibrosis.[15-19] Allogeneic stem cell transplantation offers a potentially curative treatment approach for younger patients but is associated with substantial rates of treatment-related morbidity and mortality.[20] Therefore, prognostic parameters that predict survival as precisely as possible are of clear importance in patients with MMM. A number of clinical and biological parameters have been studied; however, varying and controversial results make it difficult to establish a widely accepted scoring system. The hemoglobin level at diagnosis has been delineated as the strongest predictive parameter in these studies, but other factors such as spleen and liver size, platelet count, white blood cell count (WBC), reticulocyte count, and bone marrow histology have also been demonstrated to predict survival.[21-27] Although it is well known that the numbers of hematopoietic colony-forming cells are elevated in the peripheral blood of MMM patients, there are no studies so far addressing the potential prognostic value of this finding.

Table 1. Clinical and laboratory data of MMM patients at diagnosis.

Number	*110*
Age	68 (37–92)*
Sex, n. (male/female)	53/57
White blood cell count, $\times 10^9$/L	11.2 (1.8–83.3)*
Hemoglobin, g/dL	11.4 (4.8–17.8)*
Reticulocyte count, $\times 10^3/\mu$L	70 (3–229)*
Platelet count, $\times 10^9$/L	306 (5–2,201)*
LDH, U/L	466 (196–1940)*
Spleen size°	4 (0–16)*
Liver size°	0 (0–12)*

**Median and range; °centimeters below costal margin.*

The purpose of this study was to determine the prognostic significance of levels of circulating hematopoietic progenitor cells in patients with MMM. Our data indicate that high levels of circulating progenitor cells, when assessed in a semi-solid colony assay, are an independent risk factor for a short survival. The assessment of myeloid and erythroid progenitor cells may thus be useful in conjuction with evaluation of other risk factors in order to tailor risk-adjusted treatment strategies in MMM.

Design and Methods

Patients

A total of 110 patients who were seen at our institution between April 1990 and November 2001 were included in this study. Their diagnoses were established according to the diagnostic criteria of the Polycythemia Vera Study Group[2] and all patients were BCR-ABL negative. Nineteen patients included in this study initially presented with typical characteristics of MMM but did not have detectable splenomegaly. These patients developed spleen enlargement during the course of the disease and were reclassified as having MMM. The clinical characteristics of all patients are given in Table 1. Patients with secondary myelofibrosis, acute myelofibrosis, and myelodysplastic syndromes with myelofibrosis were excluded from the study. Peripheral blood progenitor cells were also assessed in 54 healthy subjects who served as a control group. The control group was not matched for sex and age.

Progenitor cell assessment

The number of circulating hematopoietic progenitor cells was assessed at diagnosis as described previously.[28] Briefly, peripheral blood mononuclear cells (PBMC) were isolated by centrifugation on Ficoll-Hypaque (Biochrom AG, Berlin, Germany), then the low density cells were collected from the interface between the density solution and plasma, washed twice and resuspended in Iscove's modified Dulbecco's medium (IMDM; Gibco, Paisley, UK). Subsequently PBMC were cultured in 0.8% methylcellulose, 30% fetal calf serum (FCS; Promocell, Heidelberg, Germany), 10% bovine serum albumin (Behring, Marburg, Germany), α-thioglycerol (10^{-4} mol/L) and IMDM. Cultures were supplemented with recombinant human (rh) granulocyte-macrophage colony-stimulating factor (GM-CSF) (10 ng/mL; R&D Systems, Minneapolis, USA), rh-interleukin(IL)-3 (10 U/mL; Novartis, Basel, Switzerland) and erythropoietin (EPO, 2 U/mL; Roche, Basel, Switzerland). The PBMC ($0.5\text{-}1\times 10^5$/mL) were subsequently plated in duplicate. After a culture period of 14 days at 37°C in 5% CO_2 and full humidity, cultures were examined under an inverted microscope. Aggregates with more than 40 translucent, compact, or dispersed cells were counted as CFU-GM. Bursts containing more than 100 hemoglobinized cells were counted as BFU-E. CFU-GEMMs were identified by their heterogeneous composition of translucent and hemoglobinized cells. The number of colony-forming unit-cells (CFU-GM, BFU-E and CFU-GEMM) per milliliter of blood was calculated as described elsewhere.[29] Peripheral blood $CD34^+$ cells were evaluated by flow cytometry as previously described.[30]

Statistical analysis

The median follow-up was calculated using the Kaplan-Meier method. Overall survival (OS) was calculated from date of diagnosis to last visit or date of death and investigated by plots of the Kaplan-Meier estimators. Categorical variables are described by their frequencies and percentages and compared by Fisher´s exact test. Continuous variables are described by their median and range and compared by the Mann-Whitney U-test. The effects of WBC, circulating immature myeloid cells, peripheral blast cells, hemoglobin concentration, reticulocyte count, platelet count, lactate dehydrogenase (LDH), spleen and liver size (determined by ultrasound and/or palpation), bone marrow histology (grade of cellularity, degree of bone marrow fibrosis), and the number of circulating hematopoietic progenitor cells at diagnosis were assessed as categorical and /or continuous variables, calculated by univariate and multiple Cox regression analyses and described by the resulting hazard ratio and the 95 percent confidence interval. Moreover, the prognostic value of circulating colony-forming cells

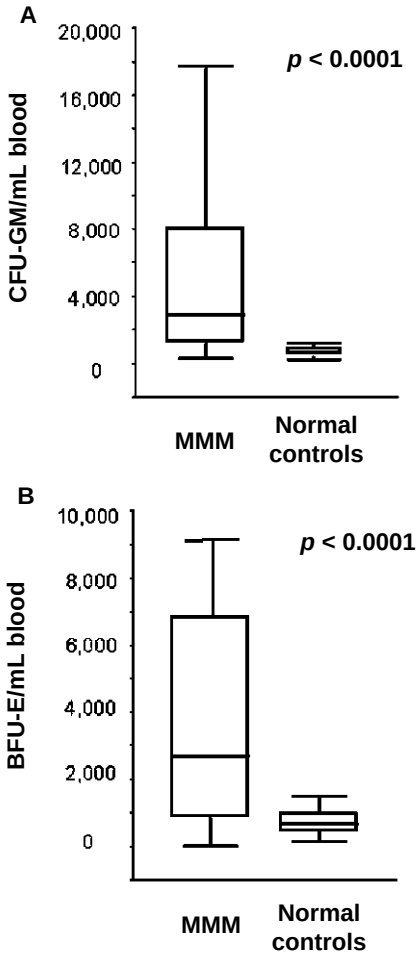

Figure 1. Comparison of myeloid and erythroid progenitor cells in peripheral blood of normal controls and patients with myelofibrosis with myeloid metaplasia (MMM). Progenitor cell numbers were assessed in semi-solid medium in the presence of IL-3, GM-CSF, and erythropoietin. The numbers of circulating CFU-GM (A) and BFU-E (B) were significantly higher among patients with MMM (n = 110) than among the controls (n = 54).

were tested in two prognostic scoring systems for MMM, namely the one proposed by Visani *et al.*,[23] in which three prognostic groups are defined based on hemoglobin (< or ≥ 10 g/dL) and immature blood granulocytes (< or ≥ 10%) and the Lille score proposed by Dupriez *et al.*,[24] which recognizes three risk groups based on two adverse prognostic factors (Hb < 10 g/L, and WBC < 4 or > 30×10^9/mL). *p*-values ≤ 0.05 were considered statistically significant. The software package SPSS 9.0 (SPSS Inc., 1999) was used for the statistical analyses.

Results

Laboratory and clinical findings in MMM patients

Table 1 summarizes the clinical and laboratory data recorded at the time of diagnosis in the study cohort of 110 patients. The median observation time was 31 months and seven patients were lost from follow-up. Median age at diagnosis was 68 years with 25 patients (23%) being below 60 years old. Immature myeloid precursors were present in 71% of the patients and 22% had more than 2% blast cells. Eighty-nine percent of the patients had LDH levels higher than the normal upper limit of 240 U/L, 83% of the patients showed spleen enlargement at diagnosis with a median size of 4 cm below the costal margin (range: 0–16 cm), and hepatomegaly was present in 45% of the patients. Of the 43 patients who died during follow up, eight deaths (19%) were due to blast transformation. According to the Lille score, there were 64 patients (58%) in the low risk group, 32 patients (29%) in the intermediate risk group, and 14 patients (13%) were placed in the high risk group. The prognostic score described by Visani *et al.* assigned 41 patients (37%) to the low risk group, 53 patients (48%) to the intermediate group, and 16 patients (15%) to the high risk group. The median numbers of circulating myeloid, erythroid and pluripotent progenitor cells were significantly higher in the study patients than in the 54 healthy controls. The median number of CFU-GM was 2,653 /mL blood (range 32–46,100), the median BFU-E 2,691/mL (0–40,800), and the median CFU-GEMM 70/mL (0–1,264) in patients with MMM (Figure 1). The control group had a median CFU-GM of 208/L blood (range 50–936), BFU-E 690/mL (120–1,862), and CFU-GEMM 20/mL (4–77). The difference between the numbers in the MMM patients and in the control group was highly significant for all three progenitor cell classes ($p < 0.0001$).

Treatment consisted solely of supportive care in 66 patients, whereas 17 patients received cytoreductive therapy with hydroxyurea or busulfan because of symptomatic splenomegaly, excessive leukocytosis or thrombocytosis. Fifteen patients participated in clinical trials receiving interferon-α or interferon-γ, and 15 patients were included in a phase 1 trial of imatinib mesylate treatment.

Influence of circulating progenitor cells on survival

The median survival of all patients was 49 months. The median number of circulating CFU-GM discriminated between patients with regards to survival. Patients with lower levels of CFU-GM showed a significantly better overall survival than did patients who had higher levels (*data not shown*). According to Kaplan-Meier analysis, there

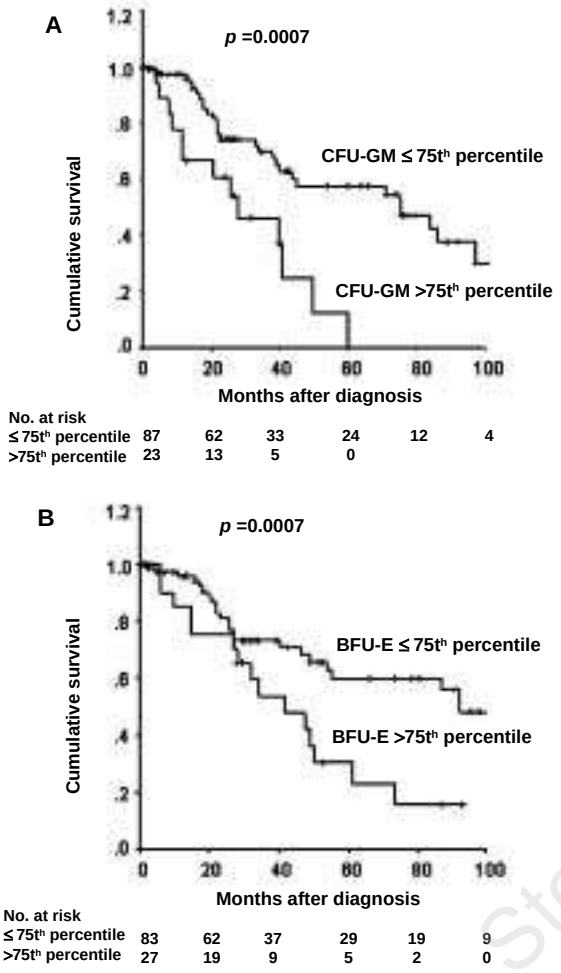

Figure 2. Cumulative survival of patients with myelofibrosis with myeloid metaplasia according to circulating CFU-GM levels and circulating BFU-E levels. Progenitor cell numbers were assessed in semi-solid medium in the presence of IL-3, GM-CSF, and erythropoietin. The median survival of patients with CFU-GM (Figure 2A) and BFU-E (Figure 2B) levels above the 75th percentile was significantly shorter than that of patients with lower levels at diagnosis.

was an even stronger divergence between median survival of patients with CFU-GM levels at or below the 75th percentile (≤ 7,533 /mL) and patients who had higher numbers of circulating CFU-GM at diagnosis (74 months and 27 months, respectively; p=0.0007) (Figure 2a). After 48 months, the probability of survival was 57% (95% confidence interval [C.I.], 43 to 71) among patients with CFU-GM levels at or below the 75th percentile, as compared to only 12% (95% C.I., 0 to 31) in patients with CFU-GM levels above the 75th percentile (Table 2A). Similar results were obtained for circulating BFU-E. The median survival of patients with

Table 2A. Circulating CFU-GM level at diagnosis and probability of survival in MMM.

Time point of observation	*CFU-GM ≤75th percentile*	*CFU-GM >75th percentile*
24 months, % (95 C.I.)	74 (63–85)	60 (39–81)
36 months, % (95 C.I.)	67 (55–79)	41 (17–65)
48 months, % (95 C.I.)	57 (43–71)	12 (0–31)

Table 2B. Circulating BFU-E level at diagnosis and probability of survival in MMM.

Time point of observation	*BFU-E ≤75th percentile*	*BFU-E >75th percentile*
24 months, % (95 C.I.)	72 (61–83)	65 (39–81)
36 months, % (95 C.I.)	69 (57–81)	46 (23–69)
48 months, % (95 C.I.)	59 (45–73)	22 (3–41)

CFU-GM: granulocyte/macrophage colony-forming unit; BFU-E: burst-forming unit-erythroid; C.I.: confidence interval.

BFU-E at or below the 75th percentile (≤ 6,911 /mL) was 74 months, whereas it was 33 months in patients with higher levels at diagnosis (p=0.007; Figure 2B). After 48 months the probability of survival was 59% (95% C.I., 45 to 73) in the patients with lower BFU-E levels as compared to only 22% (95% C.I., 3 to 41) in those with BFU-E levels in the highest quartile (Table 2B). The survival of patients with CFU-GEMM levels in the highest quartile appeared to be shorter than that of patients who had lower levels (39 and 59 months, respectively), although this difference did not reach statistical significance (p=0.12).

Influence of clinical and laboratory parameters on survival

The effects of clinical and laboratory parameters thought to correlate with survival of MMM patients were assessed by univariate regression analysis. When the numbers of circulating myeloid and erythroid progenitor cells were analyzed as continuous variables, the hazard ratio was 1.03 (95% C.I., 1.02 to 1.05, $p < 0.0001$) for each increase of 1000 CFU-GM per mL blood and 1.05 (95% C.I., 1.02 to 1.09, $p = 0.002$) for each increase of 1000 BFU-E per mL blood. Hemoglobin and age were also significantly associated with survival in our patients. Spleen size had a borderline signifi-

Table 3A. Multivariate analysis of MMM risk factors assessed at diagnosis, including CFU-GM.

	Hazard ratio	*95% C.I.*	*p value*
CFU-GM	1.03	1.01–1.04	0.0001
Hemoglobin	0.86	0.75–0.98	0.029
Age	1.04	1.01–1.09	0.012

Table 3B. Multivariate analysis of MMM risk factors assessed at diagnosis, including BFU-E.

	Hazard ratio	*95% C.I.*	*p value*
BFU-E	1.07	1.03–1.11	0.0003
Hemoglobin	0.83	0.73–0.96	0.014
Age	1.05	1.01–1.09	0.007

CFU-GM: granulocyte/macrophage colony-forming unit; BFU-E: burst-forming unit-erythroid; C.I.: confidence interval.

Table 4A. Multivariate analysis including CFU-GM in the presence of the Lille score.

	Hazard ratio	*95% C.I.*	*p value*
CFU-GM	1.03	1.01–1.04	0.002
Lille – low risk			
Intermediate risk	2.54	1.2–5.2	0.01
High risk	3.51	1.3–9.3	0.01
Age	1.05	1.02–1.1	0.005

Table 4B. Multivariate analysis including BFU-E in the presence of the Lille score.

	Hazard ratio	*95% C.I.*	*p value*
BFU-E	1.05	1.01–1.09	0.01
Lille – low risk			
Intermediate risk	2.36	1.1–4.9	0.02
High risk	3.87	1.5–9.9	0.005
Age	1.06	1.02–1.1	0.004

CFU-GM: granulocyte/macrophage colony-forming unit; BFU-E: burst-forming unit-erythroid; C.I.: confidence interval.

cance only if patients were categorized into two subgroups: patients with excessive splenomegaly (≥ 10 cm below the costal margin) and normal spleen size at diagnosis versus a moderate splenomegaly (hazard ratio 2.2; 95% C.I., 0.97 to 4.99, $p = 0.06$). All other parameters including WBC count, LDH, circulating CFU-GEMM, platelets, reticulocyte count, the percentage of immature myeloid cells and the number of blast cells in peripheral blood did not significantly correlate with survival in our study cohort.

Most patients with high levels of circulating CFU-GM also had high levels of circulating BFU-E (r = 0.516, $p = 0.01$ by Spearman's rank method). Therefore, proportional hazards analysis was performed separately for these two variables. After adjustment for age and hemoglobin, CFU-GM as well as BFU-E levels remained independent risk factors in patients with MMM (Table 3A and 3B, respectively). From a clinical point of view it is interesting to look at patients with very high numbers of circulating hematopoietic progenitor cells. We, therefore, calculated the hazard ratios associated with myeloid and erythroid progenitor cell numbers in the highest quartile.

According to the Cox regression analysis, the hazard ratio was 2.83 (95% C.I., confidence interval, 1.35 to 5.93; $p = 0.008$) for CFU-GM levels above the 75th percentile. The respective hazard ratio of patients with circulating BFU-E above the 75th percentile was 2.57 (95% C.I., 1.26 to 5.21, $p = 0.005$).

The prognostic value of circulating colony-forming cells in the presence of established risk scores

Our data clearly show the prognostic relevance of the number of circulating myeloid and erythroid colony-forming cells in patients with MMM. We next investigated whether the numbers of circulating CFU-GM and BFU-E maintain their prognostic significance in the context of established risk scores. Patients were assigned a prognostic group according to the Lille score and a risk classification as established by Visani *et al.*[23,26]

Both risk classifications clearly discriminated patients with regards to survival as assessed by Kaplan Meier survival analysis (*data not shown*). Cox regression analysis was applied to assess the influence on prognosis of circulating progenitor cells and age in the presence of the Lille score and Visani's risk classification. Table 4 shows that circulating CFU-GM and BFU-E were still independent predictors of survival in the presence of the risk groups assigned by the Lille score. When the influence of circulating colony-forming cells in the highest quartile was calculated in this mod-

el, the hazard ratio was 2.67 (95% C.I., 1.3 to 5.6; p=0.009) in patients with CFU-GM levels above the 75th percentile and 2.7 (95% C.I., 1.3 to 5.4; p=0.005) in patients with BFU-E levels in the highest quartile. Similar results were obtained when the risks groups assigned by the score of Visani *et al*. were entered into the proportional hazards analysis. Both CFU-GM and BFU-E remained significant predictors of survival, used either as continuous or as categorical variables (*data not shown*).

Comparison of levels of peripheral blood CD34$^+$ cells and circulating colony-forming cells

Recently, Barosi *et al*.[30] convincingly demonstrated that CD34$^+$ cells are elevated in peripheral blood of patients with MMM and that the absolute number of circulating CD34$^+$ cells may be used to distinguish between MMM and other forms of chronic myeloproliferative diseases. Moreover, very high numbers of circulating CD34$^+$ cells were associated with an adverse outcome in Barosi's study.[30] To elucidate a potential relation between levels of CD34$^+$ cells and colony-forming cells we simultaneously assessed both parameters in 20 patients with MMM. These results and the current treatment of patients are presented in Table 5. The median number of circulating CD34$^+$ cells in these patients was markedly higher than that in normal control subjects (*data not shown*). A significant correlation was found between the numbers of circulating CD34$^+$ cells and both the number of peripheral blood CFU-GM (r = 0.77, p < 0.001) and BFU-E (r = 0.68, p < 0.01 by Spearman's rank method). It is interesting to note that MMM patients being treated with pegylated interferon-α had significantly lower numbers of circulating progenitor cells than did untreated patients (median CD34$^+$ cells 2.7 vs. 86.4, p < 0.0001; median CFU-GM 56 vs. 5,729, p=0.001; BFU-E 235 vs. 3,023, p=0.004).

Table 5. Comparison of CD34$^+$ cells and colony-forming cells.

Pts.	*CD34$^+$ cells, $\times10^6$/L*	*CFU-GM $\times10^3$/L*	*BFU-E $\times10^3$/L*	*Current treatment*
1	652	4,522	3,023	no
2	126	1,1812	29,184	no
3	14.4	1,968	2,882	no
4	2.7	135	741	Peg-INF
5	2.3	56	169	Peg-INF
6	83.6	6,901	6,261	no
7	125.8	130	562	no
8	41.8	436	1,137	no
9	84.5	4,240	2,520	no
10	22.4	41	205	Peg-INF
11	68.1	5,729	13,195	no
12	31.5	1,690	2,627	Peg-INF
13	0.7	36	112	Peg-INF
14	1.35	53	235	Peg-INF
15	2.4	43	147	Peg-INF
16	86.4	7,080	959	no
17	293.7	11,041	15,608	no
18	4.6	1,502	2,739	anagrelide
19	21.3	2,323	2,710	Peg-INF
20	87.2	7,845	9,678	no
Median	36.7	1,829	2,573	

CFU-GM: granulocyte/macrophage colony-forming unit; BFU-E: burst-forming unit-erythroid; Peg-INF: pegylated interferon-α.

Discussion

This study shows for the first time that high levels of circulating myeloid and erythroid progenitor cells are independent risk factors for poor survival in patients with MMM. In a series of 110 consecutive patients in whom the numbers of peripheral blood progenitor cells were assessed by a semi-solid colony assay at diagnosis, circulating CFU-GM number in the highest quartile conferred a substantially worse prognosis. Similar results were obtained with the levels of circulating BFU-E. It is likely that the number of circulating myeloid and erythroid progenitor cells reflects similar biological features of MMM, namely disease activity and the proliferative capacity of hematopoietic progenitors. This may be reflected by a significant correlation between CFU-GM and BFU-E. We, therefore, analyzed their prognostic potential separately in the Cox proportional hazards analysis. Both factors turned out to be independent risk factors. Importantly, myeloid as well as erythroid progenitor cells maintained their prognostic significance when risk scores such as those established by Dupriez *et al*. and Visani *et al*. were introduced into the multivariate model. It is well established in the literature that the number of hematopoietic progenitor cells is increased in peripheral blood of patients with myeloproliferative syndromes.[8-12] Levels of circulating hematopoietic progenitor cells are particular high in MMM.[3]

Already in 1973, Paul Chervenick described increased numbers of myeloid colony forming cells in the peripheral blood of MMM patients.[8] Subsequently, this finding was extended by a number of studies which demonstrated elevated numbers of circulating erythroid, megakaryocytic, and pluripotent progenitor cells in these patients. The levels of circulating colony-forming cells were also significantly higher in MMM than in normal controls in our study. Thus, the median CFU-GM level was nearly 13 times higher, median BFU-E and CFU-GEMM approximately 4 times higher among MMM patients than in the controls. It is important to note that one patient of our study population had fewer CFU-GM than did the normal controls and in nine patients circulating BFU-E levels were lower than those in the control group. All of the nine patients with low BFU-E numbers had markedly increased CFU-GM levels. This finding is somewhat unexpected since low levels of circulating erythroid progenitor cells are typically found in the myelodysplastic syndromes (MDS).[31,32] However, the overall results of this study did not change if these patients were excluded from the analysis (*data not shown*).

Apart from circulating progenitor cells, age and hemoglobin were of independent prognostic significance, which is in keeping with literature data. The age of the patient at presentation, used as either a continuous or a categorical variable (≤ 60 and > 60 years), significantly influenced the patient's survival. However, this risk factor was not adjusted for the expected mortality in the general population. Given the advanced age of the majority of our study population, shorter survival may also reflect the mortality rate of elderly individuals. LDH is an important prognostic factor in a number of hematologic malignancies and is likely to reflect enhanced cell turnover in patients with MMM. We observed a trend towards a worse prognosis in patients with higher LDH levels but this did not reach statistical significance. Similarly, WBC count failed to be of prognostic significance. Splenomegaly has been shown to predict survival by some authors but not by others.[3-7] In our study, spleen size did not predict survival. However, a borderline prognostic significance was achieved if the patients were divided into a group with moderate splenomegaly and another group with no or excessive splenomegaly. All other potential prognostic factors analyzed in the present study including sex, immature myeloid precursors, platelet count, reticulocyte count, hepatomegaly, and bone marrow histology did not achieve prognostic significance. Roughly half of our patients received some kind of cytoreductive therapy or participated in clinical studies including treatment with interferon or imatinib mesylate. However, no study thus far has been able to demonstrate a survival benefit from such treatments.[15-19] We, therefore, do not think that these therapies have substantially affected the results of our study.

It has recently been shown that the number of $CD34^+$ cells is elevated in the peripheral blood of patients with MMM and that the absolute number of circulating $CD34^+$ cells may be used to distinguish between MMM and other forms of CMDs.[30] Similar to our results, very high numbers of circulating progenitor cells were associated with an adverse outcome. We investigated a potential relation between the number of circulating $CD34^+$ cells and colony-forming cells in a subgroup of 20 patients with primary MMM and were able to demonstrate a highly significant correlation between these two methods of progenitor cell assessment. It is interesting to note that hematopoietic progenitor levels were significantly lower in patients treated with interferon-α than in untreated controls. This finding is in clear contrast to the observation of Barosi *et al*. and may in part be explained by the limited sample size included in our subgroup analysis.[30] Flow cytometric determination of surface molecules on peripheral blood cells is easy to perform and the results can be obtained within a few hours. If our findings are confirmed in a larger cohort of patients, flow cytometry may prove useful as the preferred method of progenitor cell assessment in MMM. However, it should be pointed out that assessment of $CD34^+$ cells cannot always be directly compared with the results of *in vitro* colony assays. The numbers of circulating $CD34^+$ cells are often elevated in other hematologic malignancies, such as in myelodysplastic syndromes and acute leukemias, in which the number of colony-forming cells is usually decreased.[3]

We have recently demonstrated that the growth potential of myeloid progenitor cells in AML is critically influenced by the molecular changes that emerge from chromosomal abnormalities.[34,35] This might also be the case in MMM. Recent data, moreover, suggest a prognostic role of chromosomal changes also in patients with MMM.[36] The *in vitro* growth behavior of MMM cells may thus not only reflect the number of hematopoietic progenitor cells but also the proliferative potential of these cells. It is tempting to speculate, therefore, that a high colony growth from peripheral blood cells may indicate increased disease activity. In summary, this study shows for the first time that the numbers of myeloid and erythroid progenitor cells in peripheral blood predict survival in patients with MMM. We suggest that circulating hematopoietic progenitor cells should be assessed in these patients not only to help to establish the diagnosis but also as a new and independent prognostic parameter. More data are needed to establish which method of progenitor cell assessment more accurately defines patients who are at risk of a short survival.

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Pre-publication Report & Outcomes of Peer Review

Contributions

VS was involved in data acquisition, study design and drafting the article. EJ performed most of the laboratory work and contributed significantly to writing the manuscript. AW made substantial contributions to the overall conception and statistical analysis of the study and critically revised the manuscript for intellectual content. IS helped greatly in data acquisition and study design, FACS analysis, and revising the paper. HG performed most of the patient care, was involved in the conception of the study and critically revised the paper with regard to the intellectual content. KL and KG were both involved in study conception, interpretation of the data and drafting the article. LO was the principle investigator of this study, and thus responsible for the study design, interpretation of the data and drafting the article. All authors approved the final version of this article.

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Disclosures

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Manuscript processing

This manuscript was peer-reviewed by two external referees and by Professor Mario Cazzola, Editor-in-Chief. The final decision to accept this paper for publication was taken jointly by Professor Cazzola and the Editors. Manuscript received August 20, 2003; accepted September 24, 2003.

In the following paragraphs, Professor Cazzola summarizes the peer-review process and its outcomes.

What is already known on this topic

Using different approaches, increased numbers of circulating pluripotent and committed colony-forming cells have been found in patients with myeloproliferative disorders, suggesting abnormalities in bone marrow stem cell homeostasis in these conditions, particularly in patients with myelofibrosis with myeloid metaplasia. In a recent study in this journal, Passamonti and co-workers showed that the absolute number of circulating CD34-positive cells is lower than $15x10^6$/L in patients with uncomplicated polycythemia vera or essential thrombocythemia, while it was above this cutoff in those with myelofibrosis with myeloid metaplasia.

What this study adds

This study confirms that increased levels of circulating hematopoietic progenitor cells are found in patients with myelofibrosis with myeloid metaplasia and shows that high levels are associated with poor survival in this myeloproliferative disorder. Since evaluation of circulating CFU-GM or BFU-E is cumbersome in a clinical setting, future studies should evaluate whether flow cytometry enumeration of circulating CD34-positive cells can also provide useful prognostic information.