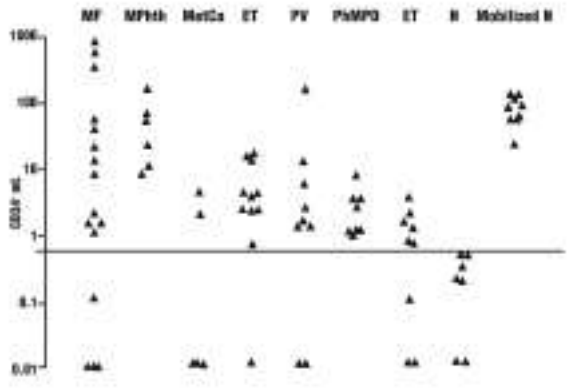

Figure 1. Circulating CD34+ cells in patients with metastatic cancer (with or without bone marrow involvement) or with various types of myeloproliferative disorders. MF: idiopathic myelofibrosis; MPhth: myelophthisis; MetCa: patients with diffuse metastatic cancer without bone marrow involvement; ET: essential thrombocythemia; PV: polycythemia vera; Ph- MPD: Philadelphia negative myeloproliferative disorders; ST: secondary thrombocytosis; N: 10 healthy subjects; Mobilized N: normal subject mobilized by granulocyte colony-stimulating factor for allogeneic donation; Solid line: upper limit in normal individuals.

abnormalities may underlie this phenomenon. A modest increase of cCD34+ cells, attributed to granulocyte-monocyte colony-stimulating factor (GM-CSF) production by the neoplastic tissue, has been described in patients with head or neck cancer.[6] We found that patients harboring cancer cells in the bone marrow had a mean of 49.2 cCD34+ cells/μL, 50 times more than in patients with metastatic cancer without bone marrow involvement. This may be the consequence of various not mutually exclusive mechanisms, including: (i) a stromal alteration, evidenced in these patients by a frequent dry tap bone marrow aspiration and some degree of fibrosis;[2-3] (ii) growth factors with mobilizing properties produced locally by the neoplastic cells; (iii) local production of molecules other than growth factors involved in stem cell mobilization. As far as concerns this last possibility, it could be relevant to note that a cleaved molecule of soluble urokinase-type plasminogen activator (uPA) receptor seems to be involved in both the metastasizing capacity of cancer cells[7] and in stem cell mobilization.[8]

Our series is too small to draw any conclusion on a possible relation between degree of cytopenia and number of cCD34+ cells; the type of primary tumor does not seem to be relevant. It is intriguing that two patients with raised cCD34+ cell counts had low hemoglobin and platelet levels but elevated neutrophil counts, suggesting a common mechanisms for high cCD34+ and white cell counts, as seen during mobilization. Several issues in this setting need to be elucidated (e.g., cytokine levels in blood and bone marrow, pattern of adhesion molecules and *in vitro* growth of cCD34+cells). From a clinical point of view, our observations suggest that high cCD34+ cell count may be indicative of bone marrow involvement in patients with metastatic cancer.

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Key words: circulating CD34 positive cells, myelophthisis, metastatic cancer.

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Red Cell Disorders

Association of the G-463A myeloperoxidase polymorphism with infection in sickle cell anemia

Infections constitute a principal cause of morbidity and mortality in sickle cell anemia (SCA). Here we present evidence to suggest that a polymorphism (G-463A MPO) in the gene encoding the myeloperoxidase (MPO) enzyme, important for the host defense system, may significantly increase susceptibility to infection in SCA.

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Sickle cell anemia is characterized by morphologically abnormal red cells, vaso-occlusion with ischemic tissue injury and susceptibility to infection. Infections, such as pneumonia, osteomyelitis, meningitis, urinary infections and septicemia, constitute a common cause of hospitalization in patients. While many patients have reduced splenic function, the mechanisms that render SCA patients more susceptible to infection are unclear. The severity of SCA varies greatly between individuals and this phenotypic variability is generally attributed to so-called genetic modulators.[1] Myeloperoxidase (MPO) is a lysosomal enzyme found in neutrophils and monocytes

Table 1. Characteristics of SCA patients included in the study.

	SCA without infections N=63	*SCA with one or more infection N=28*
Age (years)	27.8 (7.0, 51.0)	30.0 (18.0, 52.0)
Hb (g/dL)	8.2 (5.3, 13.8)	8.4 (4.8, 14.0)
Total leukocyte number $\times 10^9$/L	10.77 (5.9, 19.22)	11.34 (6.68, 15.52)
HbF (%)	6.4 (0.6, 20.3)	5.07 (0.4, 11.0)
Individuals taking hydroxyurea (20-30 kg/day) during the study	5 (7.9 %)	2 (7.1 %)
Individuals with vaso-occlusive complications	18 (28.6 %)	7 (25.0 %)

Data are represented as mean (min, max) or number of individuals (percentage). Patients were designated as having vascular complications if they presented one of the following during the clinical follow-up: ischemic stroke, priapism or necrosis of the femoral head. No statistical differences were found between the two groups for any of the characteristics (Mann-Whitney's non-parametric test or Fisher's test).

Table 2. MPO genotype and allele frequency in SCA patients not experiencing infections and in those having one or more infections during their clinical follow-up.

	Genotype frequency (%)			*Allele frequency (%)*	
	A/A	*A/G*	*G/G*	*A*	*G*
Reference Group (n=50)	3 (6.0)	17 (34.0)	30 (60.0)	23.0	77.0
SCA without infections (n=63)	2 (3.2)	20 (31.7)	41 (65.1)	19.0	81.0
SCA with one or more infections (n=28)	2 (7.1)	17 (60.7)	9 (32.1)	37.5	62.5
		p=0.0112		p=0.0096	

The reference group was formed of racially-matched healthy individuals. Fisher's exact test was used for the statistical analyses. p values refer to the comparison of the underlined groups.

and plays an important role in the host defense system, providing microbicidal activity against a number of organisms. MPO deficiency was associated with a higher occurrence of severe and chronic inflammatory processes in a group of 100 patients, compared with healthy individuals.[2] A polymorphism of the MPO gene in its promoter region (G → A, position – 463) has been shown to reduce MPO gene transcription significantly (by 25-fold) in acute myelocytic leukemia cells[4] and may modulate the incidence or severity of lung cancer,[5] periodontal disease[6] and atherosclerosis.[7]

All homozygous SCA patients (n=91, Table 1) attending the Hemoglobinopathy Clinic, UNICAMP, Brazil were included in the study. Healthy race-matched individuals were used as controls. Informed written consent was obtained from all patients and controls and the study was approved by the local ethics committee. All patients had been previously immunized against pneumococcus, *Hemophilus influenza* and hepatitis B and three patients used prophylactic antibiotics. Patients were divided into two groups; those who had not had any severe bacterial infections during their follow-up at the clinic (for a period of at least one year) and those who had had one or more severe bacterial infections, defined as the development of a bacterial infection necessitating hospitalization with antibiotic therapy. Infections identified in patients included pneumonia, sepsis, osteomyelitis, kidney infection, cellulitis, endocarditis and septic arthritis. Unconfirmed infections were not considered.

The polymerase chain reaction was utilized to amplify a 350 base-pair region surrounding the G-463A MPO polymorphism from genomic DNA, as previously described.[5] The amplification product was then sequenced. The association between infection and the MPO polymorphism was analyzed by the χ^2 test and Fisher's exact test was applied when the value of the variable was less than 5. Table 2 presents the genotype and allele frequencies for the G-463A MPO polymorphism in controls, SCA patients without infection and SCA patients who had had one or more infections. The patients without infections demonstrated a similar genotype frequency for the MPO polymorphism to that of the control population. In contrast, the patients who had had infections presented a significant increase in the frequency of the G-463A MPO genotype (p=0.0112, compared to those without infections) with an almost two-fold increase in the frequency of A/G MPO heterozygosity. The number of A/A MPO homozygous patients was too small to draw any significant conclusions regarding the frequency of the homozygous genotype; however, the allelic frequency of A was significantly higher in the group with infections than in the group without (p=0.0096). Multivariate analysis was performed to determine whether the characteristics listed in Table 1 influenced infection status, but an association was only found between the MPO polymorphism and infection. A logistic regression calculation used to take into account the time of clinical follow-up of the patients demonstrated once again that the presence of the AA or AG genotype was significantly associated with severe infection (p=0.005, OR = 3.8). These data indicate that the presence of the G-463A MPO allele appears to increase the susceptibility to infections in SCA, while patients without this polymorphism appeared to have a decreased predisposition to infection. Increased susceptibility to infection may be the consequence of a reduction in MPO production by leukocytes of affected patients and reduced anti-microbicidal function. It has been reported that phagocytic activity may be reduced in some SCA patients, since the ability of neutrophils to kill *Candida albicans* was significantly decreased in a group of steady-state SCA individuals.[8] Furthermore, the degree of impairment in the ability of neutrophils to kill *Candida spp.* correlates with the severity of the SCA.[9]

In conclusion, our data suggest that the G-463A MPO polymorphism may be a significant genetic modulator

that renders SCA patients more susceptible to infection. A multicenter prospective study may determine whether such patients should be treated with prophylatic antibiotic therapy throughout their lives to prevent infection.

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Chronic Myeloid Leukemia

Outcome of four patients with chronic myeloid leukemia after imatinib mesylate discontinuation

Imatinib mesylate (IM) therapy is effective in patients with chronic myeloid leukemia (CML). However, whether it should be discontinued in patients who achieve sustained molecular response is debated. We describe 4 patients with undetectable levels of BCR-ABL transcripts in whom IM therapy was discontinued. Two patients relapsed after 7 and 10 months and promptly responded after restarting therapy; 2 patients are off therapy at the last follow-up visit after 14 and 15 months and are still in complete molecular remission.

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Imatinib mesylate (IM) therapy leads to a complete cytogenetic response (CCyR) in the majority of patients with chronic myeloid leukemia (CML) in chronic phase. A few patients achieve complete molecular remission, defined by a four log-reduction of BCR-ABL transcripts. The negative quantitative real time polymerase chain reaction (Q-RT-PCR) is confirmed by nested PCR negativity.

Although IM therapy is effective in CML patients, some unanswered questions remain. In particular, it is unclear whether IM can actually cure CML and whether this therapy can be safely stopped in patients with complete cytogenetic and molecular responses.[1] In these patients, it is unknown whether and for how long continued therapy is required to maintain clinical, cytogenetic and molecular responses. Between 2000 and 2004, we treated 88 CML patients with IM: 62 were interferon-α (IFN) pre-treated patients in late chronic phase and 26 were newly- diagnosed patients in early chronic phase. Sixty-three patients (71.5%) achieved a CCyR and in 15 of them (24%) BCR-ABL transcripts became undetectable.

Here we describe the cytogenetic and molecular outcome of 4 patients with CML in whom IM therapy was discontinued after the achievement of a complete molecular response in the bone marrow and peripheral blood. In all cases, IM was discontinued because of the patients' requests and not because of toxic effects. The patients' characteristics are shown in Table 1. All patients were pre-treated with IFN; only patient #2 was in CCyR and was switched to IM because of IFN-intolerance. No patient had a family donor for allotransplant or was a candidate for an unrelated transplant. During IM therapy at 400 mg/day, no patient required dose reduction or discontinuation due to hematologic or non-hematologic toxicity. At the time of IM withdrawal, all patients had been in sustained CCyR for 17 to 30 months and in complete molecular response for 13 to 19 months. All patients showed normal cell morphology on bone marrow examination and none of them had additional cytogenetic abnormalities.

The relative quantification of BCR-ABL transcripts was performed by Q-RT-PCR using 1 μg of total RNA, isolated by an RNeasy mini kit (Qiagen) from 10^7 Ficoll-hypaque separated mononucleated cells, reverse-transcribed as previously described.[2] Relative quantification