Hematopoietic Stem Cells

High number of circulating CD34⁺ cells in patients with myelophthisis

Six patients with bone marrow micrometastases from solid cancers presented with increased numbers of circulating CD34⁺ cells; the CD34⁺ cell counts were very high in some cases. By contrast, no patient with metastatic cancer without bone marrow involvement showed raised numbers of circulating hemopoietic progenitors.

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The circulating CD34 (cCD34⁺) cell count is assessed daily to monitor stem cell mobilization in patients undergoing stem cell harvest from peripheral blood. Another condition with raised numbers of cCD34⁺ cells is idiopathic myelofibrosis,[1] a finding suggesting that a normal bone marrow microenvironment is essential for the binding of CD34⁺ cells to stroma. Myelophthisis is a peculiar bone marrow microenvironment alteration caused by the presence of a high number of non-hematopoietic neoplastic cells, impairing hematopoiesis by largely unknown mechanisms. Space occupation is not the main mechanism, since micrometastases do not colonize the whole marrow; rather, marrow is usually hypocellular, with some degree of fibrosis revealed at bone biopsy.[2-3] We investigated the number of cCD34⁺ cells in patients with myelophthisis.

We studied eleven patients with diffuse metastatic cancer (Table 1). Myelophthisis was suspected in patients presenting uni-, bi-, or trilineage cytopenia not due to chemotherapy, and was documented by the finding of non-hematopoietic neoplastic cells in a bone marrow aspirate and/or biopsy. Peripheral blood, collected in K_3EDTA, was processed within 3 hours of venipuncture. CD34⁺ cells were detected by a combination of two monoclonal antibodies, fluorescein isothiocyanate-conjugated anti-CD45 and phycoerythrin-conjugated anti-CD34 (Becton Dickinson, Boston, MA-USA); c-kit expression was also measured. Fifty microliters of blood were incubated with the antibodies for 20 min in tubes containing a known number of microbeads. Cells were identified by FACSCalibur (Becton Dickinson, Boston, MA, USA) using scattering and fluorescence methods, and analyzed following the sequential gating strategy recommended by the International Society for Hematotherapy and Graft Engineering (ISHAGE). To exclude any influence of drugs, patients were studied before or at least three months after chemotherapy; none of the patients had received growth factors.

Of the eleven patients with metastatic cancer of various origin, six showed bone marrow micrometastases, suggested by cytopenia and documented by bone aspirate and/or biopsy, while five patients had no sign of bone marrow involvement. Marrow fibrosis (surrounding the metastatic lesions) was detected in the three patients with myelophthisis who underwent bone biopsy. cCD34⁺ (c-kit⁺) cell counts were elevated in all 6 patients with myelophthisis, and were particularly high in four.. In contrast, CD34⁺ cells were almost undetectable in the patients with metastatic cancer without bone marrow involvement (Table 1). The mean number of cCD34⁺ cells was 49.2 and 1.2 cells/μL in the two groups, respectively. Among patients suffering from various types of disorders and tested for cCD34⁺ cells in our institution, the number of cells detected was second highest in myelophthisis; the highest number was found in idiopathic myelofibrosis (Figure 1).

Stem cell mobilization by growth factors and/or chemotherapy is caused by modifications of membrane-bound molecules that enable detachment of cells from the stroma. The only disease in which a high number of cCD34⁺ cells has been described in the absence of any treatment is idiopathic myelofibrosis;[1,4,5] both an altered bone marrow microenvironment and stem cell surface

Table 1. Circulating CD34⁺cells in metastatic cancer patients with or without bone marrow involvement.

	Case					Blood count					
	Age	Gender	Type of cancer	Hb g/dL	WBC $\times10^9$/L	N $\times10^9$/L	Plt $\times10^9$/L	Cancer cells in bone marrow	Previous therapy	CD34+ /μL
1	46	F	Breast	8.2	4.400	2.200	63	yes	None	60.6
2	34	M	Lung	7.9	5.310	3.240	163	yes	Cis-platinum	19.4
3	53	F	Stomach	8.4	17.000	14.620	49	yes	None	48.2
4	66	M	Lung	7.7	14.600	10.366	24	yes	None	149.4
5	53	M	Stomach	8.2	3.040	1.824	70	yes	None	8.1
6	50	F	Breast	9.4	15.500	7.500	51	yes	None	9.7
7	71	M	Stomach	11.1	6.410	4.295	265		5-FU	0
8	64	M	Lung	14.7	7.790	6.465	199		Taxotere	4.0
9	59	M	Lung	13.4	9.920	6.448	191		Cis-platinum	2.0
10	77	M	Colon	13.8	3.930	2.974	95*		5-FU	0
11	64	M	Stomach	11.9	8.600	6.450	237		5-FU	0

**: low count due to hypersplenism secondary to hepatitis C virus-related chronic hepatitis.*

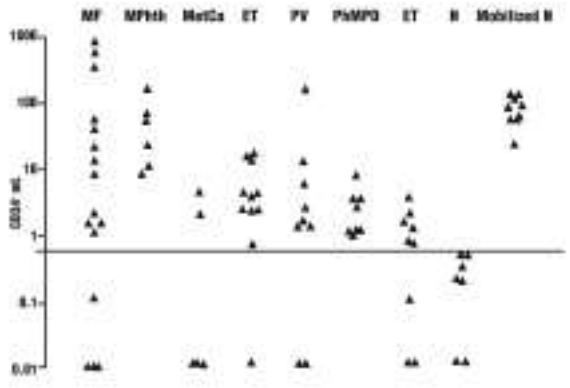

Figure 1. Circulating CD34+ cells in patients with metastatic cancer (with or without bone marrow involvement) or with various types of myeloproliferative disorders. MF: idiopathic myelofibrosis; MPhth: myelophthisis; MetCa: patients with diffuse metastatic cancer without bone marrow involvement; ET: 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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References

1. Barosi G, Viarengo G, Pecci A, Rosti V, Piaggio G, Marchetti M, et al. Diagnostic and clinical relevance of number of circulating CD34+ cells in myelofibrosis with myeloid metaplasia. Blood 2001;98:3249-55.
2. Cotelingam JD. Bone marrow biopsy: interpretative guidelines for the surgical pathologist. Adv Anat Pathol 2003;10:8-26.
3. Bauermeister DE. Quantitation of bone marrow reticulin: a normal range. Am J Clin Pathol 1971;56:24-31.
4. Andreasson B, Swolin B, Kutti J. Patients with idiopathic myelofibrosis show increased CD34+ cell concentrations in peripheral blood compared to patients with polycythaemia vera and essential thrombocythemia. Eur J Haematol 2002;68: 189-93.
5. Passamonti F, Vanelli L, Malabarba L, Rumi E, Pungolino E, Malcovati L, et al. Clinical utility of the absolute number of circulating CD34-positive cells in patients with chronic myeloproliferative disorders. Haematologica 2003;88:1123-9.
6. Pandit R, Lathers DM, Beal NM, Garrity T, Young MR. CD34+ immune suppressive cells in the peripheral blood of patients with head and neck cancer. Ann Otol Rhinol Laryngol 2000; 109:749-54.
7. De Bock CE, Wang Y. Clinical significance of urokinase-type plasminogen activator receptor (uPAR) expression in cancer. Med Res Rev 2004;24:13-39.
8. Selleri C, Montuori N, Visconte V, Carriero MV, Sidenius N, Serio B, et al. Involvement of the urokinase-type plasminogen activator receptor in hematopoietic stem cell mobilization. Blood 2005;105:2198-205.

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