



Figure 1. Platelet counts and treatments.

correct the platelet level (Figure 1). Finally, intravenous bolus of high dose methylprednisolone were administered, inducing a slight and transient increase of platelet rate. At that time, the patient refused any other treatment and did not have a follow-up. As for the CD, clinical improvement was observed regarding disappearance of diarrhea and a Crohn's disease activity index of less than 50, compared with 298 at diagnosis (CDAI < 150: quiescent phase; 150 < CDAI < 450: acute attack; CDAI > 450: very severe);⁵ ulcerative inflammation of the duodenal cap persisted and CD granulomas were found on gastric, ileal and colonic biopsies. Anemia responded to iron supplementation.

Anemia is a frequent finding in CD patients, mainly due to iron deficiency (as a result of chronic intestinal bleeding, iron malabsorption, or impaired dietary intake) and chronic inflammation, or to cobalamin and/or folate deficiencies or inadequate erythropoietin production.⁶ Humoral and cellular immune mechanisms contribute to the onset of chronic inflammatory bowel diseases (CD and ulcerative colitis). Chronic T-lymphocyte activation, abnormalities in the production of γ interferon and α tumor necrosis factor which affect B-cell proliferation and differentiation into immunoglobulin secreting cells, infiltration of plasma cells into mucosa with increased local production of IgG have been reported in CD patients.^{7,8} Association of chronic inflammatory bowel diseases with autoimmune cytopenias might be more than coincidental and account for the same immune dysregulation. At least five cases of ITP have been reported in patients with ulcerative colitis.⁹ Whatever the relationship between CD and ITP in our patient, co-existence of these two disorders complicated their respective clinical courses. Corticosteroids and γ -globulins have been shown to reduce bowel inflammation in some patients with CD;¹⁰ initially administered to treat severe

thrombocytopenia, they induced clinical improvement of CD but failed to correct platelet rate in our patient.

Key words

Idiopathic thrombocytopenic purpura, Crohn's disease

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More on the appropriate fluorochrome-conjugated CD34 antibody choice for the flow cytometric detection of circulating progenitor cells

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We have collected data showing that the phycoerythrin (PE)-conjugated 8G12 (HPCA-2) CD34 MoAb allows an increased flow cytometric resolution of small number of circulating CD34⁺ hematopoietic cells.

Table 1. CD34⁺ cell number in PB and leukaphereses samples estimated by means of PE-, FITC-HPCA-2, and PE-pool-anti-CD34⁺ MoAbs.

Samples	(A) PE-HPCA-2	(B) FITC-HPCA-2	(C) PE-POOL (Immu-133, Immu-409, Qbend-10)	p values A vs B / B vs C / A vs C
PB %	0.81±0.97 (0.01-4.9)	0.71±0.9 (0.001-4.7)	0.75±0.93 (0.002-4.8)	< 0.0001 / < 0.0001 / < 0.0001
/μL	46.4±81.8 (0.22-616.4)	40±74.9 (0.04-581.5)	42.9±77.8 (0.07-589.6)	< 0.0001 / < 0.0001 / < 0.0001
LK %	2.63±1.77 (0.58-6.2)	2.31±1.74 (0.55-6)	2.54±1.75 (0.56-6.1)	< 0.0001 / < 0.0001 / < 0.0001
/μL	2084.5±2138.3 (446-9300)	1951.3±2073.4 (423.5-8850)	2025.4±2113.3 (431-9150)	< 0.0001 / < 0.0001 / < 0.0001

Data are expressed as mean ± standard deviation and range in brackets.

In a recent issue of *Haematologica*, Ortuño *et al.* focused on the important differences in phycoerythrin (PE)- or fluorescein-isothiocyanate (FITC) directly conjugated anti-CD34 monoclonal antibody (MoAb) used to detect more accurately the number of circulating progenitor cells after mobilizing therapy in cancer patients.¹ In fact, their work showed that by using 8G12 (HPCA-2) Class III anti-CD34 MoAb, significantly higher values were observed in PE-CD34⁺ cells when compared with FITC-CD34⁺ cells both in leukaphereses (LK) and in peripheral blood (PB) samples.

In our experiments for the clinical estimation of circulating CD34⁺ cells, we used the Milan Protocol as described by Siena *et al.*,²⁻⁴ in which a directly FITC-conjugated HPCA-2 anti-CD34 MoAb is required. However, the PE-conjugated anti-HPCA-2 MoAb seem to further increase the resolution between cytometrically CD34⁺ and CD34⁻ cells. In addition, there exist other classes of anti-CD34 MoAbs, which are based on the differential sensitivity to enzymatic cleavage with glycoprotease. In order to establish what kind of MoAb should be preferred in a routine estimation of CD34⁺ cells, we carried out a study on 118 PB and 22 LK samples from 11 patients with hematological malignancies and who were undergoing mobilizing therapy. Briefly, three 50 μL aliquots of whole blood or appropriately diluted LK samples were placed in each tube with 5 μL of the following MoAbs: a) FITC-CD34 (HPCA-2), from Becton Dickinson (BD), San José, CA, USA; b) PE-CD34 (HPCA-2), from BD; c) PE-Pool-CD34, from Immunotech, Marseille, France. The latter is a blend of 3 PE-conjugated MoAbs, all directed to CD34 antigen and belonging to the Class I (Immu-133 and Immu-409) and Class II (Qbend-10) MoAbs. Samples were processed and analyzed as previously described.⁵ Table 1 shows the results obtained. Statistically significant differences (paired t-test) were found among the 3 groups of samples tested with the dif-

ferent MoAbs. The highest values of CD34⁺ cell number were obtained by using PE-conjugated-HPCA-2 MoAb, while the lowest by using FITC-conjugated-HPCA-2 MoAb both in PB and LK samples. Finally, Class I and Class II anti-CD34 MoAbs blend gave intermediate values.

In conclusion, our study clearly indicate that, because of the small number of CD34⁺ PBPCs that can be detected, the PE-conjugated 8G12 (HPCA-2) CD34 MoAb should be preferred, resulting in an increased flow-cytometric resolution.

Key words

CD34, flow cytometry, monoclonal antibodies

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