Waldenström’s macroglobulinemia complicated with acute myeloid leukemia.  
Report of a case and review of the literature

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The evolution of Waldenström’s macroglobulinemia (WM) into chronic or acute myeloid leukemia (AML) is a rare event. Most of these cases have occurred after treatment with alkylating agents. We herein report a case of WM terminating in an acute myelomonocytic leukemia after treatment with prednimustine and chlorambucil and present a review of the literature.

An 80-year-old woman was admitted to the Hospital three years ago due to recurrent urinary tract infections. Physical and analytical examination showed abnormal results only in a urea of 72 mg/dL, a serum protein level of 68 g/L; Hb 76 g/L, Plt 23 × 10^9/L, and increased IgM levels (0.96 g/L) with a monoclonal IgM-k band. The bone marrow aspirate disclosed 88% mature vacuolated lymphoid cells. WM was diagnosed and prednimustine (20 mg/day) was started and maintained for 9 months, after which it was discontinued due to the development of psychotic symptoms. At this moment, the blood counts and the serum immunoglobulin levels were normal with a persisting monoclonal IgM-k band. Chlorambucil (5 mg/day) was then started and maintained for 16 months, at which time, due to her optimal clinical status, treatment was discontinued. Ten months later, anemia and thrombocytopenia were observed: Hb 72 g/L; WBC 7 × 10^9/L (42% neutrophils; 9% lymphocytes; 13% monocytes; 2% eosinophils; 2% basophils; 32% blasts); Plt 8 × 10^9/L. The blast cells fell into two groups: 60% were monoblasts while 40% presented no differentiation. Immunophenotype of blast cells was CD34+, HLA-DR+, CD38+, CD13+, CD14+. The bone marrow aspirate showed moderate dysplastic changes with an increased number of promyelocytes, myelocytes, monocytes and promonocytes (25%), 30% blasts with the same characteristics as those observed in peripheral blood and 15% of lymphoplasmocytic cells. Acute myelomonocytic leukemia (FAB subtype M4) was diagnosed. Treatment with low doses of Ara-C (10 mg/m²/12h) was administered for 21 days without response. Further therapy was refused by the patient’s family and she died one month later from progressive disease.

A review of the literature (Medline®, January 1966-December 1996) disclosed only 22 cases of WM terminating in AML (Table 1). Including our case, five of the 23 cases compiled had not been previously treated for their WM, while the remaining had received alkylating agents for varying periods of time (median 21 months; range 2-125). Prednimustine, a combination of the alkylating agent chlorambucil and prednisolone, has never been reported in previous papers describing WM terminating in AML, and it has rarely been reported as producing secondary malignancies. Although in those patients who received treatment for their WM, AML secondary to this therapy seems to be the most widely accepted etiological theory; the possible etiology of the non-treated cases remains controversial. The first theory postulates that leukemia may have arisen from a differ...
ent clone of cells either secondary to the same etiologic agent that produced the WM or secondary to another etiologic factor, the simultaneous occurrence of the two processes being coincidence. In the case reported by Ligorsky et al., a decrease in the IgM levels was observed simultaneously with the reduction of blasts; the author speculates the possibility that this IgM was produced abnormally by blasts. This circumstance has not been confirmed in subsequent cases.

All subtypes of AML according to the FAB classification, except M3 and M5 have been reported. Although different degrees of cytopenia have been reported as preceding the development of AML in 9 cases, the simultaneous existence of myelodysplasia was reported in only 2 cases. A decrease in the serum IgM levels could not predict the development of AML, as has been suggested by several authors in other secondary neoplasms following WM (only present in 1 of the 9 patients in which this data was available). Response to treatment is poor and the survival is very short.

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Key words
Waldenström macroglobulinemia, acute myeloid leukemia

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References

Idiopathic thrombocytopenic purpura associated with Crohn’s disease
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Besides cytopenia related to treatment, several hematological disorders such as anemia, abnormal platelet activity, thrombosis, presence of anticaldolipin or anti-neutrophil antibodies, cyclic neutropenia, and myelodysplasia, have been reported in patients with Crohn’s disease (CD). The case we report here is the first one documenting the association of idiopathic thrombocytopenic purpura (ITP) with CD.

A 19-year-old woman was referred to our department for microscopic hypochromic anemia (Hb: 7.2 g/dL, MCV: 55 µm³, MCHC: 29%) and thromboctopenia (20×10⁹/L).

At admission, physical complaints consisted of fatigue, diarrhea (3 to 4 stools a day) and weight loss (6 kg in 6 months); physical examination was normal. Biological findings, including bone marrow aspiration smears analysis, led to the diagnosis of ITP associated with iron-deficiency anemia. No antplatelet antibodies were detected. Upper gastrointestinal endoscopy and full colonoscopic examination disclosed ulcerative inflammation of the duodenal cap, polypoid lesions and linear ulcerations on the terminal ileum. Histopathologic analysis of large bowel and ileum biopsies found a lymphocytic inflammatory infiltrate of the mucosa associated with granulomas composed of epithelioid and giant cells consistent with the diagnosis of CD. Successively, standard dose prednisolone, intravenous γ-globulins, and splenectomy failed to
correct the platelet level (Figure 1). Finally, intra-
venous 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 fol-
low-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: quies-
cent phase; 150<CDAI<450: acute attack;
CDAI>450: very severe); 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 inade-
quate erythropoietin production. Humoral and
cellular immune mechanisms contribute to the
onset of chronic inflammatory bowel diseases (CD
and ulcerative colitis). Chronic T-lymphocyte acti-
vation, abnormalities in the production of γ inter-
feron and α tumor necrosis factor which affect B-
cell proliferation and differentiation into immu-
noglobulin 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
two 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, initially administered to treat severe
thrombocytopenia, they induced clinical improve-
ment of CD but failed to correct platelet rate in our
patient.

**Key words**
Idiopathic thrombocytopenic purpura, Croh’s disease

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**References**
2. Chamouard P, Grunebaum L, Wiesel ML, et al. Prevalence and significance of anticardiolipin antibod-
4. Sahay R, Prangnell DR, Scott BB. Inflammatory bowel disease and refractory anemia (myelodyspla-
5. Best WR, Becktel JM, Singleton JW. Rederived values of the eight coefficients of the Crohn’s disease activi-
7. MacDonald TT, Murch SH. Aetiology and patho-
8. Van Dullemen HM, van Deventer SJ, Hommes DW,
et al. Treatment of Crohn’s disease with anti-tumor necrosis factor chimeric monoclonal antibody
9. Fernandez-Miranda C, Mateo S, Kessler, Gonzalez-
Castello J. Immune thrombocytopenia and ulcer-
10. Bellanger J, Cosnes J, Gendre JP, Beaugerie L, Mala-
fosse M, Le Quintrec Y. Traitement de la maladie de
Crohn de l’adulte. Rev Med Interne 1994; 15:676-
89.

**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 phycoery-
thin (PE)-conjugated 8G12 (HPCA-2) CD34 MoAb allows an increased flow cytometric resolution of
small number of circulating CD34+ hematopoietic cells.
In a recent issue of Haematologica, Ortuño et al. focused on the important differences in phycoery-thrin (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. 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., 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, Qbend-10) and Class II MoAbs. Samples were processed and analyzed as previously described.

Table 1 shows the results obtained. Statistically significant differences (paired t-test) were found among the 3 groups of samples tested with the different 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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**References**