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

JUAN NICOLÁS RODRÍGUEZ, ANTONIO FERNÁNDEZ-JURADO, MARÍA LUZ MARTINO, DALMIRO PRADOS

Servicio of Hematología, Hospital "Juan Ramón Jiménez", Huelva, Spain

The evolution of Waldenström's macroglobulinemia (WM) into chronic or acute myeloid leukemia (AML) is a rare event.[1] 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 developement 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 $CD34^+$, $HLA\text{-}DR^+$, $CD38^+$, $CD13^+$, $CD14^+$. 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/m^2/12h) 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).[2-10] 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.[11] 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.[1] The first theory postulates that leukemia may have arisen from a differ-

Table 1. Characteristics of the patients reported in the literature (including ours).

Case	Sex (M/F)	Age	Treatment	Interval (months)	Duration therapy (months)	FAB subtype	MDS
1	F	19	NO	0	0	NA	NA
2	F	53	NO	0	0	NA	NA
3	F	78	Chl	NA (weeks)	NA (weeks)	NA*	NA
4	NA	NA	Chl	NA	NA	NA	NA
5	F	66	Mel	72	72	M6	NO
6	F	80	NO	0	0	M4	NO
7	M	81	Mel	42	42	M6	NO
8	F	68	Chl	36	21	NA (sug.M2)	YES
9	M	69	NO	11	0	M4	NO
10	F	68	NO	48	0	M4	YES
11	M	42	Chl, Mel, Cy, Vinc	168	123	NA (sug.M2)	NO
12	M	71	Cy	54	49	M4	NO
13	M	52	Vinc, Mel	16	16	M4	NO
14	M	63	Chl	54	41	M4	YES
15	M	79	Chl	9	2	M2	NO
16	M	70	Chl, Cy	72	43	M1	NO
17	M	85	Chl, Cy	29	29	M2	NO
18	M	69	Chl	24	5	undifferent	NO
19	M	75	Mel, PChT	34	21	NA	NO
20	M	54	Mel	48	19	NA	NO
21	M	62	Chl	84	21	M4	YES
22	F	73	Chl, Cy, PChT	125	125	M7	NO
23	F	80	Prm, Chl	36	25	M4	YES

*Case #23 is the present case. M/F = Male/Female; Interval = Interval between WM diagnosis and the apparition of AML; MDS = Existence of previous myelodysplasia or at diagnosis of AML; NA = Not available; *Stem cell leukemia; Sug. M2 = Suggesting M2; Undifferent. = Undifferentiated leukemia. Chl = Chlorambucil; Mel = Melphalan; Cy = Cyclophosphamide; Vinc= Vincristine; PChT = Polychemotherapy; Prm = Prednimustine.*

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.*,[8] both macroglobulinemia and leukemia cells contained the same immunoglobulin; a possible explanation for this could be that blast cells engulf the paraprotein secreted by the plasma cells. Finally, as in the case reported by Allen[6] 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 preceeding the developement of AML in 9 cases, the simultaneous existence of myelodysplasia[12] was reported in only 2 cases. A decrease in the serum IgM levels could not predict the developement of AML, as has been suggested by several authors in other secondary neoplasms following WM[5] (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.

Acknowledgments
We thank Dr. Joan Bladé from the Postgraduate School of Hematology "Farreras Valentí", Hospital Clínic (Barcelona) for his help in the critical review of the manuscript.

Key words
Waldenström macroglobulinemia, acute myeloid leukemia

Correspondence
Juan Nicolás Rodríguez Rodríguez, Servicio de Hematología, Hospital "Juan Ramón Jiménez", 21005 Huelva, Spain. Fax international +34.59.202080.

References

1. Rosner F, Grünwald HW. Multiple myeloma and Waldenström's macroglobulinemia terminating in acute leukemia. Review with emphasis on karyotypic and ultrastructural abnormalities. NY State J Med 1980; 80:558-70.
2. Majumdar G, Slater NGP. Waldenström's macroglobulinemia terminating in acute myeloid leukemia: report of a case and review of the literature. Leuk Lymphoma 1993; 9:513-6.
3. Tangün Y, Saracbasi Z, Inceman S. Akut miyeloblastik losemi ve makroglobulinemi: 2 nci vak'a (hematolojik remisyonla protein anomalisinin duzelmesi). Trk Tip Cemiy Mecm 1968; 34:126-30.
4. Videbaek A. Unusual cases of myelomatosis. Br Med J 1971; 2:326.
5. Mackenzie MR, Fundenberg HH. Macroglobulinemia: an analysis of 40 patients. Blood 1972; 39:874-89.
6. Allen EL, Metz EN, Balcerzak SP. Acute myelomonocytic leukemia with macroglobulinemia, Bence Jones proteinuria, and hypercalcemia. Cancer 1973; 32:121-4.
7. Cardamone JM, Kimmerle RI, Marshall EY. Development of acute erythroleukemia in B-cell immunoproliferative disorders after prolonged therapy with alkylating drugs. Am J Med 1974; 57:836-42.
8. Ligorsky RD, Axelrod AR, Mandell GH, Palutke M, Prasad AS. Acute myelomonocytic leukemia in a patient with macroglobulinemia and malignant lymphoma. Cancer 1977; 39:1156-62.
9. Casciato DA, Scott JL. Acute leukemia following prolonged cytotoxic agent therapy. Medicine 1979; 58:32-47.
10. García F, Aporta R, Cañizares F, Cabrera A. Leucemia aguda megacarioblástica en el curso de una macroglobulinemia de Waldenström. Sangre 1996; 41:162-3.
11. Mahe M, Raffi F, Grolleau JY, Badout S. Leucémie aiguë monoblastique au cours d'un lymphome malin non Hodgkinien traité par prednimustine. Therapie 1989; 44:378-9.

Idiopathic thrombocytopenic purpura associated with Crohn's disease

MARION BAUDARD,* THIERRY MOLINA,° KADER BENFIGUIG,# JEAN-PIERRE BETHOUX,@ ROBERT ZITTOUN*

**Service d'Hématologie Clinique; °Laboratoire d'Anatomie-Pathologie; #Service de Médecine Interne et Hépato-Gastro-Entérologie; @Service de Chirurgie Générale, Hôpital Hôtel-Dieu, Paris, France*

Besides cytopenia related to treatment, several hematological disorders such as anemia, abnormal platelet activity, thrombosis, presence of anticardiolipin or anti-neutrophil antibodies, cyclic neutropenia, and myelodysplasia, have been reported in patients with Crohn's disease (CD).[1-4] 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 microcytic hypochromic anemia (Hb: 7.2 g/dL, MCV: 55 μm^3, MCHC: 29%) and thrombocytopenia (20×10^9/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 antiplatelet 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

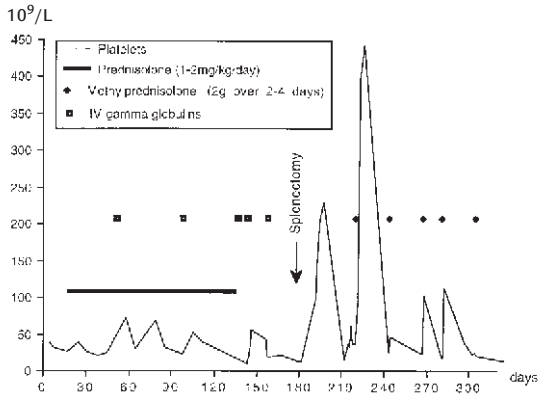

Figure 1. Platelet counts and treatments.

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 colinic 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, Croh's disease

Correspondence
Robert Zittoun, Service d'Hématologie Clinique, Hôpital Hôtel-Dieu, 1 place du Parvis Notre Dame, 75004 Paris, France. Phone international +33.42.348413. Fax international +33.42.348406.

References

1. Schaufelberger HD, Uhr MR, McGuckin C, et al. Platelets in ulcerative colitis and Crohn's disease express functional interleukin-1 and interleukin-8 receptors. Eur J Clin Invest 1994; 24:656-63.
2. Chamouard P, Grunebaum L, Wiesel ML, et al. Prevalence and significance of anticardiolipin antibodies in Crohn's disease. Dig Dis Sci 1994; 39:1501-4.
3. Lindgren S, Floren CH, Lindhagen T, et al. Low prevalence of anti-neutrophil cytoplasmic antibodies in ulcerative colitis patients with long-term remission. Eur J Gastroenterol Hepatol 1995; 7:563-8.
4. Sahay R, Prangnell DR, Scott BB. Inflammatory bowel disease and refractory anemia (myelodysplasia). Gut 1993; 34:1630-1.
5. Best WR, Becktel JM, Singleton JW. Rederived values of the eight coefficients of the Crohn's disease activity index (CDAI). Gastroenterology 1979; 77:843-6.
6. Gasché C, Reinisch W, Lochs H, et al. Anemia in Crohn's disease. Importance of inadequate erythropoietin production and iron deficiency. Dig Dis Sci 1994; 39:1930-4.
7. MacDonald TT, Murch SH. Aetiology and pathogenesis of chronic inflammatory bowel disease. Baillière Clin Gastroenterol 1994; 8:1-34.
8. Van Dullemen HM, van Deventer SJ, Hommes DW, et al. Treatment of Crohn's disease with anti-tumor necrosis factor chimeric monoclonal antibody (cA2). Gastroenterology 1995; 109:129-35.
9. Fernandez-Miranda C, Mateo S, Kessler, Gonzalez-Castello J. Immune thrombocytopenia and ulcerative colitis. J Clin Gastroenterol 1994; 18:85-6.
10. Bellanger J, Cosnes J, Gendre JP, Beaugerie L, Malafosse 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

Giovanni D'Arena, Mario Carotenuto

Hematology Division, IRCCS "Casa Sollievo della Sofferenza" Hospital,San Giovanni Rotondo, Italy

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, thee 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 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

Correspondence
Giovanni D'Arena, MD, Hematology Division, IRCCS "Casa Sollievo della Sofferenza" Hospital, 71013 San Giovanni Rotondo, Italy. Phone international +39.882.410539; fax international +39.882.411705.

References

1. Ortuño F, Ferrer F, Lozano ML, Heras I, Moraleda JM, Vicente V. Differences in phycoerythrin- or fluorescein-isothiocyanate conjugated 8G12 on CD34⁺ cell evaluation. Haematologica 1997; 82:334-5.
2. Siena S, Bregni M, Brando B, et al. Flow cytometry for clinical estimation of circulating hematopoietic progenitors for autologous transplantation in cancer patients. Blood 1991; 77:400-9.
3. Siena S, Bregni M, Brando B, et al. Flow cytometry to estimate circulating hematopoietic progenitors for autologous transplantation: comparative analysis of different CD34 monoclonal antibodies. Haematologica 1991; 76:330-3.
4. Cell estimation in peripheral blood for autografting in patients with cancer. In: Hematopoietic stem cells. The Mulhouse Manual. Alpha Press, USA; 1994. p. 23-30.
5. D'Arena G, Scalzulli P, Musto P. Circulating hematopoietic progenitor cells in leukapheresis: flow cytometric estimation [letter]. Haematologica 1996; 81: 95-6.