

IgG₃ type. The hematic and serous blood group was identified as 0 Rh positive; anti-DNA and ANA antibodies were negative.

Viral serology demonstrated IgM⁺ anti-varicella antibodies, anti-HIV, CMV, infectious mononucleosis and hepatitis serology was negative. An immunohematological study carried out at the regional transfusion center showed the 3+/4+ positivity for the direct Coombs' test with the antibody type corresponding to an IgG₃. A search for allo-antibodies was negative. Elution of the autoantibody showed anti-DC specificity (concentration <1/128). The patient's genotype, determined by saline monoclonal sera, was CDe/CDe. Variants of the D antigen were ruled out using a panel of anti-D monoclonal sera.

The patient required a transfusion of two concentrates of O group Rh negative erythrocytes. Cross tests were negative in the saline and Coombs' phase. The transfusion was carried out under intensive care, without provoking an acute hemolytic reaction. Corticoid treatment at a dose of 2 mg/kg/day was begun and produced a progressive rise in the hemoglobin (14 gr/dL). At that time, the patient still showed slight hemolysis making it necessary to continue corticoid treatment. Abdominal CT documented the persistence of moderate splenomegaly and erythrokinetics evidenced shortened average erythrocyte survival (12 days) with a pattern of splenic sequestration.

Splenectomy was performed and the patient's clinical course has been satisfactory. He currently maintains a normal hemoglobin level with no treatment at all.

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Unusual evolution to immunoblastic lymphoma of a case of Waldenström macroglobulinemia presenting with thrombocytopenia

Sir,

Waldenström macroglobulinemia (WM) is a lymphoproliferative disease characterized by the secretion of elevated quantities of monoclonal IgM; approximately 6% of cases evolve to immunoblastic lymphoma (IL).^{1,2} On the other hand, the association of lymphoproliferative processes with thrombocytopenia is well known.³ We report a 65-year-old woman who came to our service with mucocutaneous bleeding and thrombocytopenia as the initial manifestations of WM which rapidly evolved to IL. At presentation the patient showed hepatosplenomegaly, platelets $14 \times 10^9/L$, leukocytes $6.9 \times 10^9/L$ with normal differential count, and hemoglobin 10 g/dL. Immunoglobulin quantification was: IgG 1141 mg/dL, IgA 151 mg/dL, and IgM 333 mg/dL, with a normal immunoelectrophoretic pattern. Anti-platelets antibodies (IgG and IgM) were strongly positive. Marrow aspiration revealed abundant megakaryocytes and peripheral blood immunophenotype showed 30% CD19⁺, CD22⁺, Dr⁺, FMC7⁻ lymphocytes, positive surface immunoglobulins with λ monoclonality (++) , CD5⁺, CD25⁺, and CD10⁻. Steroid treatment was begun with only a partial response, platelets remained at $100 \times 10^9/L$ for four months, after which they fell to $10 \times 10^9/L$. Abdominal CT was normal, as were new

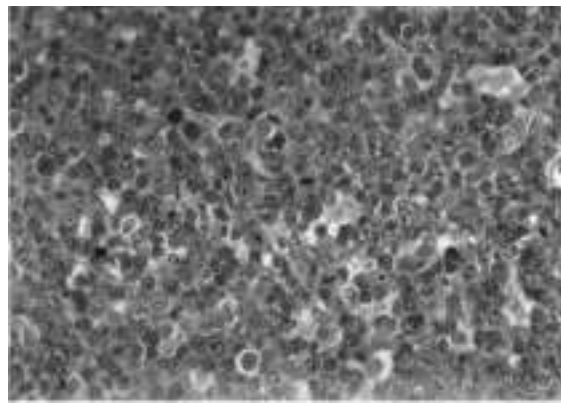


Figure 1. Microscope examination of a cervical peripheral lymph node (H.E. 400x): immunoblastic lymphoma (polymorphic immunocytoma). Diffuse proliferation of lymphoplasmacytoid cells and large cells with numerous mitoses are shown.

marrow aspiration and repeated immunophenotyping. This is difficult to explain except for the possibility that it was caused by the lympholytic action of the steroids or by focal bone marrow involvement. Splenectomy was performed and histological examination revealed a lymphoplasmacytoid/immunocytoma lymphoma with λ monoclonality. Following splenectomy there was a partial response and for a few months the platelet level was maintained, after which another abdominal CT scan revealed low density hepatic lesions with internal adenopathies. Immunoelectrophoresis demonstrated a monoclonal IgM peak of 2 g/dL and Bence Jones proteinuria with monoclonality.

Six months later the IgM had risen to 10 g/dL and marrow aspiration showed 10% plasma cells, some of which were binucleated, and a minimum of lymphoid infiltration in some zones. At this time the platelet count normalized. Treatment with cycles of chlorambucil and prednisone was begun along with plasmapheresis. There was no change in the amount of the IgM and a monoclonal IgG peak (2 g/dL) also appeared. A few weeks later numerous peripheral adenopathies developed and a biopsy of one of these revealed an immunoblastic lymphoma with λ monoclonality (Figure 1). The patient died of pulmonary hemorrhage twenty months after diagnosis. Several aspects stand out in this case: the appearance of thrombocytopenia before WM; its disappearance when the monoclonal peak rose (for which we have no explanation); the rapid evolution to IL with identical monoclonality, as reported by others,⁴ and the appearance of monoclonal IgG along with the monoclonal IgM at the end of the disease evolution. To our knowledge, only one other such case has been reported previously.⁵

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A rapid and sensitive methods for the analysis of von Willebrand factor multimeric structure

Sir,

Human von Willebrand factor (vWF) mediates platelet adhesion and thrombus formation under high shear stress conditions; moreover, it binds and stabilizes procoagulant factor VIII in circulating blood.¹ Quantitative and qualitative vWF abnormalities lead to a congenital bleeding disorder: von Willebrand disease (vWD). Mature vWF circulates in plasma as a series of multimers ranging in size from 450 kDa to in excess of 10,000 kDa.¹ The multimeric structure of vWF can be demonstrated in normal subjects and in patients with different types of vWD, with the exception of the severe form of the disease.² Multimeric analysis of plasma and platelet vWF is widely used as a diagnostic tool in screening and characterizing vWD; moreover, it is useful in the quality control of therapeutic plasma concentrates containing vWF.³

We report a rapid, sensitive method for multimeric analysis of vWF. The procedure is based on vertical SDS-agarose mini-gel electrophoresis, followed by electroblotting and alkaline phosphatase immunostaining. Electrophoresis was performed on a vertical mini-gel apparatus (Mini-Protean II, Bio-Rad, Hercules, CA, USA) using the discontinuous buffer system of Ruggeri and Zimmerman.⁴ Separating gel consisted of 1.1% (low resolution) or 2.6% (high resolution) low gelling temperature agarose; stacking gel consisted of 0.8% high gelling temperature agarose. Citrated plasma was diluted 1:10 in sample buffer⁴ and incubated at 60° for 15 min; 10 µL samples were

applied to the wells. Electrophoresis was carried out at 60V for 20 min and then was continued for 6h at a constant 35V. Electrophoresis buffer was composed of 50 mM Tris, 384 mM glycine, 2 mM EDTA, 0.1% SDS, pH 8.35; the buffer temperature was held at 16°C. Gels were then transferred onto nitrocellulose filters by overnight blotting at 450 mA (50 mM phosphate buffer, 0.04% SDS, pH 7.4). After blocking non-specific sites, filters were incubated in anti-vWF primary antibody (Dakopatts, Glostrup, Denmark) at 4°C for 3h. The membranes were then washed and incubated in alkaline phosphatase-conjugated anti-rabbit IgG (ICN, Costa Mesa, CA, USA) at 4°C for 2 h. Finally, vWF multimers were visualized by using BCIP/NBT as chromogenic substrate. The good transfer efficiency allowed us to obtain a normal pattern of more than 20 multimers by low resolution analysis (Figure 1), thus facilitating differential diagnosis between type 1 and most forms of type 2 vWD. High resolution analysis (Figure 1) separated the structure of the smallest vWF multimers into five bands. This gel was suitable for characterizing type 2 vWD samples. A total working time of about 36h is needed to complete multimeric analysis; on the other hand, a minimum of 5 days is needed when multimer visualization is performed by autoradiography. Moreover, the disadvantages and hazards of handling radioactive materials are avoided. Compared to the horizontal method, the vertical electrophoretic system enabled us to optimize the buffer-gel contact and to prevent agarose drying. There is no fading of nitrocellulose filters developed by the alkaline phosphatase substrate; dried filters can be scanned by a densitometer and stored. Finally, the mini-gel system minimizes reagent consumption.

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Figure 1. Immunoenzymatic staining of plasma vWF multimers by low resolution (LR) and high resolution (HR) analysis.

1. normal plasma; 2. type 1 vWD; 3. type 1 vWD after DDAVP infusion; 4. type 2A vWD; 5. type 2B vWD; type 3 vWD; 7. normal plasma. Arrowheads indicate the start of separating gel (cathode).

CD5/CD19/CD23 chronic lymphocytosis

Sir,

the characterization of the chronic lymphoid leukemias is based on cell morphology and immunological markers.^{1,2} Difficulties in establishing defining criteria for each disease derive from the existence of overlapping features among the various disorders and a degree of variability in morphology, histology and phenotypic profile within the same entity. We report a case of B-cell lymphoproliferative disorder with a potentially confusing phenotype.

A 48-year-old woman was admitted to our Hospital for anemic syndrome. Four years earlier she had been diagnosed as suffering from chronic lymphocytic leukemia (CLL) for an isolated peripheral blood lymphocytosis of $42 \times 10^9/L$. Immunophenotype performed at that time disclosed a positivity for CD5, CD19 and CD23. Bone marrow biopsy showed a diffuse infiltration by mature lymphocytes with 12% plasma cells. Marked splenomegaly with a monoclonal IgM paraprotein