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. 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. 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. 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.

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. 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 buffer and incubated at 60°C 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 µA (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 2h. 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 3h 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.

References


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CD5/CD19/CD23 chronic lymphocytosis

Sir,

the characterization of the chronic lymphoid leukemias is based on cell morphology and immunological markers. 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 x 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