Immunostaining of von Willebrand factor multimers on agarose gels and nitrocellulose filters

Human von Willebrand factor (VWF) multimeric analysis is commonly performed by agarose gel electrophoresis, electroblotting, and immunoenzymatic staining; however, high molecular weight (HMW) multimers are poorly transferred on nitrocellulose and should be visualized by direct gel staining with radiolabeled anti-VWF antibody and autoradiography or luminography.

von Willebrand factor (VWF) is synthesized in the form of an extra high molecular weight (HMW) polymer. A limited cleavage by a metalloprotease down-regulates the size of plasma VWF to a series of lower molecular weight multimers.[1,2] The largest multimers are the most effective in promoting platelet adhesion and aggregation under the high wall shear conditions that characterize blood flow in the microcirculation;[3,4] when HMW multimers are absent, as occurs in some variants of von Willebrand's disease (VWD), bleeding occurs.[4]

We describe here an immunoenzymatic method for visualization of VWF multimers directly on agarose gel, and compare it with immunostaining of VWF blotted onto nitrocellulose. Electrophoresis was carried out as previously described,[5] then gels were divided into halves. One of them was fixed onto a plastic film, rinsed 2×20 min in 20 mM Tris, 500 mM NaCl, pH 7.4 (TBS), before overnight incubation in polyclonal anti-VWF antibody, then washed 6× in 0.05% Tween 20-TBS (TTBS), and incubated 2 h in alkaline phosphatase-labeled secondary antibody. After additional 6× washing in TTBS, VWF multimers were visualized by using BCIP/NBT as chromogenic substrate. The other part of the gel was electroblotted onto nitrocellulose,[5] then immunostained with the gel procedure, except that 5×3 min washing steps were used. Densitometric scanning resolved about 20 distinct multimers on the gel and filter (Figure 1); however, a wider spectrum of HMW multimers hardly resolvable in distinct bands was shown on the gel (Figure 2A). Multimers larger than the tenth (>4,500 kDa) represented 24.2% of the overall multimeric distribution on nitrocellulose, and 36.8% on the agarose gel (Figure 2B); moreover, multimers larger than the fifteenth (about 6700 kDa) were 5.3% of total on the filter, and 15.2% on the gel. Budde *et al.* used a luminographic detection technique to assess the relative proportion of HMW multimers in normal individuals:[6] they found 15-25% VWF multimers larger than the tenth. This result is comparable with that which we obtained by filter scanning, but significantly lower than the result of our gel analysis. The described gel immunostaining procedure is simple to perform and requires only the instrumentation for electrophoresis; supporting agarose by a plastic film avoids gel breakages as a consequence of the extensive washings. The alkaline phosphatase-based immunostaining is sensitive enough to detect HMW multimers, without the need for radiolabeled antibody followed by autoradiography[7] or luminography,[6] or the avidin-biotin system as an immunoenzymatic enhancer.[8] Thus, the method may be suitable to characterize qualitative VWD variants with supranormal VWF multimers, such as type 2M Vicenza,[9] and to reveal a deficient activity of VWF-cleaving protease in patients with chronic relapsing thrombotic thrombocytopenic purpura.[10]

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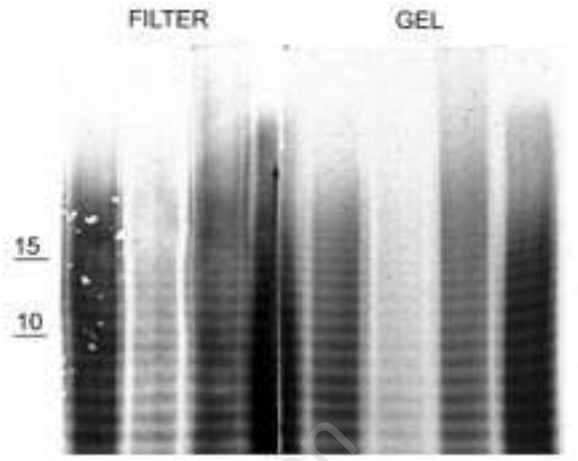

Figure 1. Immunostaining of VWF multimers on nitrocellulose filter and agarose gel. Lanes 1 and 4: normal plasma; 2: type 1 VWD; 3: type 1 VWD after desmopressin infusion, showing extra HMW multimers. The arrow indicates the dye front; the tenth and the fifteenth multimers are marked.

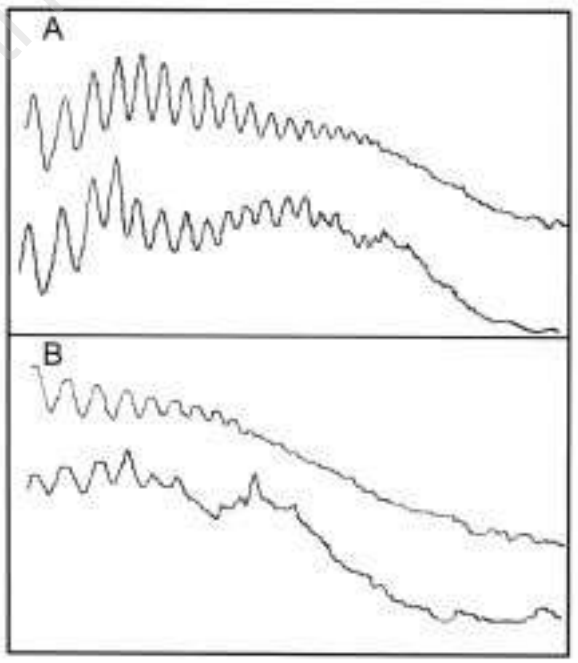

Figure 2. Densitometric scanning of the overall multimer spectrum (A) and of multimers larger than the tenth (B), of lane 3. The upper scanning of each pair is referred to agarose gel, the lower to nitrocellulose filter.

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