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### Abnormalities of plasma von Willebrand factor multimeric structure induced by extracorporeal circulation

Sir,

Using an immunoblotting technique, we analyzed the multimeric structure of plasma von Willebrand factor (vWF) after extracorporeal circulation (ECC) in patients undergoing cardiovascular surgery intervention. An abnormal vWF structure, similar to that observed in type 2A von Willebrand disease, was demonstrated in 7 out of 12 patients. This finding induces us to evaluate vWF function further in these patients.

Extracorporeal circulation is a prerequisite for cardiovascular surgery. However, repeated passage of the patient's blood through the cardiopulmonary bypass results in contact activation of blood, which leads to the initiation of the clotting cascade, complement activation, fibrinolytic mechanisms and kinin release; moreover, platelet function is impaired and leukocyte activation occurs (including neutrophil degranulation and protease release, oxygen radical production, and the synthesis of cytokines by mononuclear cells).<sup>1,2</sup>

To assess the validity of blood recovery after ECC in pediatric cardiovascular surgery, we studied the multimeric structure of plasma vWF in 12 patients (4 to 26 yrs, mean 11.6) with congenital heart disease undergoing cardiovascular surgery. ACD-anticoagulated blood was withdrawn before ECC. No blood transfusions were given during ECC, and none of the patients had significant hemorrhagic or thrombotic diathesis after ECC. Immediately after ECC we collected the residual blood in the circuit to study vWF. vWF multimeric analysis was performed as described elsewhere.<sup>3</sup> Briefly, plasma samples were electrophoresed on a mini-gel system (1.1% and 2.6% LGT-agarose for

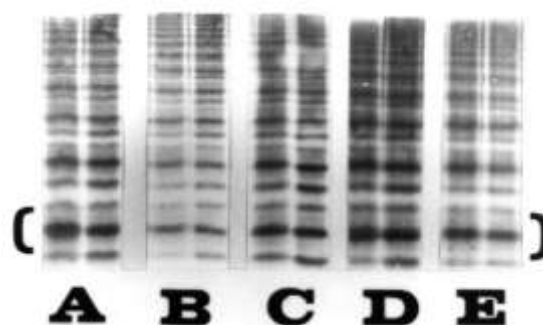


Figure 1. High resolution vWF multimeric analysis of plasma of 5 patients is shown, before and after ECC (left and right lane of each pair, respectively). The increased prominence of the faster satellite band of each multimer is evident. Brackets indicate the lowest molecular weight multimer.

low and high resolution analysis, respectively), then gels were transferred onto nitrocellulose filters by electroblotting. Filters were incubated with anti-vWF antibody, then with an alkaline phosphatase-labeled secondary antibody; finally, vWF multimers were visualized using a chromogenic substrate, and filters were scanned by a densitometer.

An identical spectrum of vWF multimers was assessed before and after ECC in all patients by low resolution analysis. Before ECC, high resolution analysis demonstrated a normal composition of individual vWF multimers in all patients; on the other hand, an abnormal multimeric structure was detected in 7 patients after ECC: the abnormal pattern always consisted of increased prominence of the faster sub-band of each multimer (Figure 1), closely resembling that occurring in type 2A von Willebrand disease (vWD).<sup>4</sup> vWF is synthesized by megakaryocytes and endothelial cells in the form of very large polymers of 225-kDa subunits. However, circulating vWF undergoes proteolysis under physiologic conditions in order to prevent pathological formation of platelet thrombi.<sup>5</sup> An abnormal susceptibility to proteolysis leads to abnormal vWF multimeric structure in some patients affected by type 2 vWD.<sup>6,7</sup>

In our experience, the finding of a normal spectrum of vWF multimers after ECC excluded significant loss of the largest multimers caused by adsorption to the ECC circuit surface.

Tsai *et al.*<sup>8</sup> demonstrated that shear stress in capillary tubings may increase vWF proteolysis by enhancing the proteolytic activity in plasma, and by causing conformational changes in the vWF molecule itself, which render the cleavage site more accessible to plasma proteases. It is probable that both shear stress in the ECC circuit and an increase of the overall proteolytic activity of plasma could be responsible for the

finding of an abnormal structure of plasma vWF in 7 out of 12 patients undergoing ECC. vWf function may, however, be impaired in these patients; this fact should be assessed if residual blood is recovered after ECC.

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### **Key words**

*von Willebrand factor, multimeric analysis, extracorporeal circulation.*

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