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PLASMA THROMBOMODULIN CONCENTRATIONS IN INFANTS AND CHILDREN UNDERGOING CARDIAC CATHETERIZATION

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ABSTRACT

Circulating plasma thrombomodulin (TM) is an endothelial cell marker which may reflect endothelial injury. To find out to what extent diagnostic cardiac catheterization irritates vascular endothelium we conducted a prospective study in 91 children. Soluble TM concentrations, along with thrombin generation, were measured before, at the end of and 24 hours after cardiac catheterization. Compared to starting values, TM concentrations showed a clearly significant increase at the end of cardiac catheterization and returned to pretreatment values 24 hours later. Thrombin generation followed a similar pattern. Five out of the 91 children demonstrated resistance to activated protein C (APCR). With respect to the remaining 86 children, all five APCR cases showed increased thrombomodulin concentrations along with enhanced thrombin generation. Data from this study indicate that increased TM concentrations after cardiac catheterization in children are a sign of short-term endothelial damage. Furthermore, together with enhanced thrombin generation, elevated plasma concentration of soluble TM may reflect this receptor's possible anticoagulant properties.

Key words: thrombomodulin, thrombin generation, APC- resistance, pediatric cardiac catheterization

Circulating plasma thrombomodulin (TM) is an endothelial cell marker which may reflect endothelial injury. TM acts as a thrombin receptor that neutralizes the fibrin-forming effect of thrombin and accelerates the formation of the anticoagulant protein C/S pathway.[1] TM therefore belongs to the anticoagulant defense system against thrombosis. Increased TM levels have been described in various diseases such as ARDS, thromboembolic vascular insults, TTP, diabetes, LE and hyperhomocysteinemia, reflecting alterations of the vascular system at the endothelial level.[2-7] To find out to what extent diagnostic cardiac catheterization irritates vascular endothelium we conducted a prospective study in infancy and childhood.

Materials and Methods

Ninety-one children (neonate-16 years) underwent percutaneous venous cardiac catheterization with low-dose heparin[8] during a 12-month period. Blood samples for coagulation studies were obtained by separate venous punctures immediately before, at the end of, and 24 hours after cardiac catheterization, drawn into premarked 3 mL plastic tubes (citrate 3.8%/blood ratio 1:10; Saarstedt®) and centrifuged at 3000g and 4° C. Platelet poor plasma was snap frozen (–70° C), stored at –70°C and serially investigated in duplicate six weeks to three months later. Plasma TM concentrations were analyzed with the ELISA technique (Stago, Asnières-sur-Seine, France); thrombin generation (prothrombin fragment F1+2) was measured with Enzygnost® F1+2 micro (Behring Werke, Marburg, Germany). In addition, to evaluate the influence of inherited thrombophilia in all children, resistance to the anticoagulatory effect of activated protein C (APCR), protein C, protein S, factor V and antithrombin were studied as described earlier.[9]

Calculations of medians and ranges and non-parametric statistics (Wilcoxon signed rank)

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were performed with the Apple computer (Macintosh Performa 630) StatView 4.02 program.

Results

The results (median; median absolute deviation: MAD) are shown in Figures 1 and 2.

Compared with starting values, TM concentrations showed a clearly significant ($p<0.01$) increase at the end of cardiac catheterization and returned to pretreatment values 24 hours later (Figure 1). Thrombin generation (F1+2; Figure 2) followed a similar pattern; a clearly significant increase was observed at the end of catheterization, followed by normalization 24 hours later.

Moreover, no correlations were found between increased thrombin generation or TM concentrations and age, weight or duration of catheterization.

Five out of the 91 children (5.49%) showed resistance to activated protein C: four were heterozygous carriers (+/-) of the factor V Leiden mutation in the factor V gene and one child was found to be homozygous (+/+). No further protein C, protein S, factor V or antithrombin deficiencies were found in the remaining 86 children.

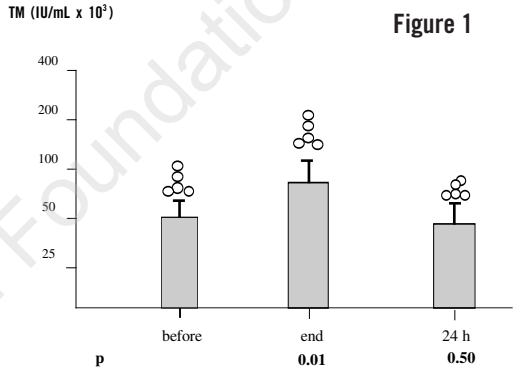
Interestingly, compared to the median TM concentrations of the 86 patients without inherited thrombophilia, all five children with the factor V Leiden mutation presented increased TM concentrations and enhanced thrombin generation prior to and 24 hours after the procedure (Figures 1 and 2: single values).

Discussion

Data from this study indicate that increased thrombomodulin concentrations measured in peripheral blood immediately after cardiac catheterization in infancy and children, followed by normalization within 24 hours, are a sign of short-term endothelial damage. Furthermore, together with enhanced thrombin generation, elevated plasma concentration of soluble TM may reflect this receptor's possible anticoagulant properties.

It has been shown recently that TM is an important regulator of activated thrombin that converts thrombin from a procoagulant to an anticoagulant by altering its substrate specificity so that it activates protein C.[10] Moreover, due to its associated chondroitin sulphate moiety, TM is also involved in the heparin interaction, thus accelerating thrombin inhibition by antithrombin.[1]

In the present study, all five APCR children showed enhanced thrombin generation along with basally increased plasma TM concentrations with respect to infants without APCR. In addition, we have just confirmed these prelimi-



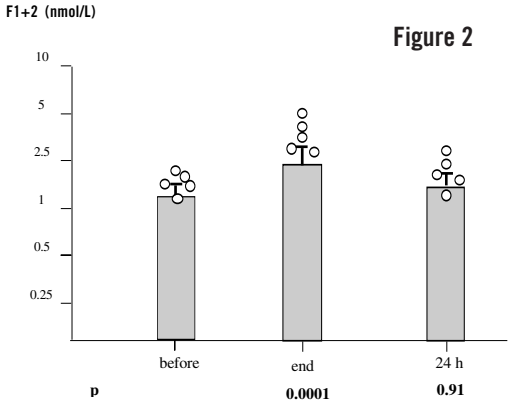


Figure 1. Soluble thrombomodulin values (median and MAD; single values of APC-resistant children) immediately before, at the end of, and 24 hours after cardiac catheterization. Compared to starting values, TM showed a significant ($p<0.01$) increase at the end of the procedure, with normalization 24 hours later.

Figure 2. Thrombin generation (F1+2: median and MAD; single values of APCR-resistant children) immediately before, at the end of, and 24 hours after cardiac catheterization. Compared to starting values, F1+2 showed a significant ($p<0.0001$) increase at the end of the procedure, with normalization 24 hours later.

nary findings in a series of 46 APCR children; compared to age-matched healthy controls we found significantly increased thrombin generation and increased plasma TM concentrations.

Besides the anticoagulant properties discussed, elevated plasma TM concentrations may reflect a loss of endothelial thrombomodulin, which might be expected to lead to decreased protein C activation. However, in the pediatric population studied no protein C deficiencies were found. Thus the hypothesis that enhanced plasma TM reflects a loss of endothelial TM is not compatible with the normal protein C plasma values observed in the children investigated.

Concerning literature data, neither age, weight, duration of catheterization nor the use of ballon catheters was found to be a risk factor for late venous thrombosis following pediatric cardiac catheterization.[11,12] In agreement, no correlations between enhanced plasma values of thrombin generation or TM concentrations were observed in the present study.

In conclusion, with regard to TM being a potent inhibitor of coagulation activation,[1] the present data might be interpreted as a counter-regulatory mechanism; enhanced thrombin generation due to decreased capacity to inactivate factor Va in APCR children may lead to increased TM values, thus maintaining the coagulation balance.

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