SPECIFIC ASSAYS OF HEMOSTASIS PROTEINS: FACTOR VII •

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Several prospective studies have shown that, together with hyperlipidemia, obesity, hypertension and smoking, hypercoagulability may be considered a risk factor for coronary heart disease and stroke[3-5, 10]. The Northwick Park Heart Study suggested an important role of factor VII (FVII) in the arterial thrombotic disease of heart and brain. Elevated plasma levels of FVII were found to be associated with cardiovascular disease and subsequent cardiovascular-related death, as well as with serum cholesterol and triglycerides[4, 5, 9]. Further studies confirmed that patients at high risk of cardiovascular disease have either increased levels of FVII and FVII procoagulant activity or increased FVII mass[1, 2, 6, 7].

From these studies it appears that FVII may be involved in the natural history of thrombotic artery disease, so that its measurement should be performed as a routine test to better evaluate the thrombotic risk. Nevertheless, there are many different factors which influence FVII assay and should be considered in order to obtain a good standardization of the test. Among these factors, the thromboplastins and the FVII-deficient plasmas used to carry out the test may affect the results. For this reason, the CISMEL Hemostasis Subcommittee undertook a project on FVII standardization.

MATERIALS AND METHODS

The FVII assay was performed using a one-stage clotting method employing a photooptical coagulometer (Coag-A-Mate X2).

Pooled plasma from 50 healthy subjects (31 men and 19 women) was diluted in imidazole buffer and assayed at 5 dilutions ranging from 1:10 to 1:160 to obtain the reference curve. FVII assays were carried out using all combinations of the 5 thromboplastins and 6 FVII-deficient plasmas reported in tab. 1.

Key-words: Factor VII; Risk factors; Standardization.

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RESULTS AND DISCUSSION

The first aim of our study was to verify the reproducibility of the test. The between-assay precision was assessed for each FVII-deficient plasma/thromboplastin combination by testing each dilution of the calibration curve on 8 different occasions.

The coefficient of variation (CV) of the clotting time was always less than 7% for 4 out of 5 thromboplastins at each of the considered dilutions, whereas the bovine thromboplastin showed a CV of about 20%. Moreover, the CV values did not appear to be affected by the different FVII-deficient plasmas when used with the same thromboplastin.

The second aim of our study was to evaluate the possible influence of different FVII-deficient plasmas and different thromboplastins on FVII clotting assay. For this purpose we compared all the slopes of the calibration curves obtained with the different combinations of FVII-deficient plasmas and thromboplastins. The statistical analysis showed that there were no differences between the slopes of the regression lines obtained with the different FVII-deficient plasmas when used with the same thromboplastin.

On the other hand, we noticed a significant influence on the slopes of different thromboplastins when used with the same FVII-deficient plasma. In fact, three thromboplastins (namely 1, 2 and 4) showed no difference in slope when compared among themselves, whereas the remaining two (namely 3 and 5) showed different slopes when compared firstly among themselves and, secondly, to the others. These findings mean that while the slope of the calibration curve is not affected when different plasma substrates are used, the thromboplastins influence the value of the slope[8]. For this reason, the identification of the thromboplastin which could give the best performance in testing FVII is quite important for the standardization of the method.

According to this viewpoint, while the type of FVII-deficient plasma used for the clotting test is of no importance, the thromboplastin must be accurately chosen.

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thromboplastins

1. lyophilized, human placentas (Behring Institute)
2. non-lyophilized, rabbit brain (Manchester Comparative Reagents)
3. lyophilized, rabbit brain (Organon Teknika Corp.)
4. lyophilized, rabbit brain (Ortho Diagnostic Systems)
5. lyophilized, bovine brain (Instrumentation Labs)

FVII-deficient plasmas

A. quick-frozen, from a patient congenitally deficient in FVII
B. lyophilized, from patients congenitally deficient in FVII (Merz-Dade AG)
C. lyophilized, from patients congenitally deficient in FVII (Organon Teknika Corp.)
D. lyophilized, artificially depleted from normal plasma (Behring Institute)
E. lyophilized, artificially depleted from normal plasma (Boehringer Mannheim GmbH)
F. lyophilized, artificially depleted from normal plasma (Ortho Diagnostic Systems)

Tab. 1 - Thromboplastins and FVII-deficient plasmas. *(All the plasmas considered had a residual activity of <1%).*

SUMMARY

Factor VII (FVII) activity should be measured in order to evaluate the risk for coronary artery disease. The measurement of FVII by means of a standardized clotting method seems to be influenced by the thromboplastins used, while the FVII-deficient plasmas do not affect the results.

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