

## Spuriously Low Levels of Protein C with a Protac® Activation Clotting Assay

Dear Sir,

Protein C (PC) plasma activity is now widely determined in patients who have experienced thrombotic events. Several methods for PC activity measurements have been published and they were compared in a multicenter trial (1). A common feature is the need for an extraction step. More recently an alternative method using direct activation of PC by a snake venom (Protac®) has become commercially available (2, 3), which avoids the tedious extraction step and the thrombin neutralization procedure. Activated PC is then determined either by clotting assay in an aPTT system or with a chromogenic substrate. The main advantages of clotting assays lie in their ability to use the natural substrate (FVIIIa) of PC (and hence to detect some variants with normal amidolytic activity) and to be less sensitive to acetylated forms of PC (4, 5).

In some patients without anticoagulant treatment we have found discrepant results between Protac® based clotting and chromogenic assays (reagents, Standard Human Plasma and Fibrintimer from Behringwerke, Marburg, FRG), as previously reported by Löbermann et al. (3). As shown in the table the values obtained with the chromogenic assay were higher and better correlated with antigen levels measured by electroimmunoassay (Assera Plate Proteine C, Stago, Asnières, France) than those found with the clotting assay ( $r = 0.975$  versus  $0.309$ ). However, when the clotting assay was performed at higher plasma dilutions (1/20 and 1/40) than that recommended by the manufacturer (1/10) the PC levels were found to increase and the correlation with PC antigen values to improve ( $r = 0.92$  at 1/40 dilution), even if the correction was not complete.

A common feature in this group of patients was a high level of FVIII activity (determined by one-stage clotting assay, reagents from Behringwerke). The apparent low PC levels and their partial correction by higher plasma dilutions could arise from an increase in the total FVIII concentration due to patient plasma FVIII contribution in the assay mixture.

The influence of FVIII on the clotting assay was also tested by measuring PC levels after DDAVP infusion, since its effect on haemostasis is relatively specific for the FVIII/von Willebrand complex and t-PA (6). The raise in FVIII (mean three-fold over baseline values) did not change PC levels when measured with the chromogenic assay but gave low values with the clotting one, which could not be totally corrected by increasing dilutions (data not shown). PAI was undetectable in DDAVP plasma (Coa-Set t-PA/PAI, KabiVitrum, Sweden). DDAVP and Normal Human Plasma were also submitted to extraction according to Francis and Patch (7) (except that the MES-citrate-NaCl supernatant was immediately diluted 1/10 in the Veronal buffer supplemented with 0.1 mol/l HEPES pH 7.6), and clotting activity after Protac® activation was measured. Extraction did abolish the dilution effect, suggesting that some plasma component, probably FVIII, is the cause of the artefact.

Thus, the Protac® clotting method alone cannot be recommended for screening PC deficiency. The partial correction by

Table Protein C values

Patient	FVIII	PC Ag	PC chrom	PC clotting, dilutions		
				1/10	1/20	1/40
1	230	150	140	36	88	100
2	370	215	184	26	56	168
3	300	126	131	62	86	100
4	322	72	68	18	36	48
5	185	126	121	50	92	100
6	185	122	93	60	70	80
7	322	136	108	49	56	72
8	260	130	136	26	52	72
9*	248	31	36	0	0	25
10	200	92	94	60	81	82

Results are expressed in %.

\* Protein C deficient patient.

dilution has to be considered only as an indicator of some artefact. Therefore every sample whose value is below the normal range has to be assayed with an immunologic, and eventually, with an additional chromogenic method (3). When discrepant values are obtained the extraction procedure should be performed.

Philippe de Moerloose  
Guido Reber  
Claude A. Bouvier  
Haemostasis Unit  
Geneva University Hospital  
1211 Geneva 4, Switzerland

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