

## ANTICOAGULANT MECHANISMS IN SURGICAL PATIENTS

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**Key words:** anticoagulant mechanisms, protein C, protein S, surgical patients

**Abstract:** Protein C (PC) and its cofactor protein S (PS) are potent physiological anticoagulant mechanisms. As thrombotic events are known to be a major complication of the surgical procedures, we studied the behavior of these anticoagulant mechanisms in surgical patients. PC:Ag level was significantly decreased ( $63,3 \pm 4,2$ ,  $p < 0,001$ ) in 29 critically - ill surgical patients when compared to 32 healthy control subjects. When compared to 10 controls subjects, PS:Ag was also significantly decreased ( $59,2 \pm 4,96$ ,  $p < 0,01$ ) in 12 surgical patients in critical condition. These changes could be explained by the switch of the hepatic protein synthesis during the acute phase reaction developing in critically ill surgical patients towards the increased production of acute phase proteins, while reducing the secretion of PC and PS, cholinesterase and albumin. These observations emphasize the risk for thrombosis in postoperative states and stress the importance of a thorough investigation of hemostasis in surgical patients.

### INTRODUCTION

Blood coagulation is regulated by several anticoagulant mechanisms. One of these is represented by the protein C system (3). Protein C is a vitamin K dependent serin protease secreted by the liver in an inactive form, which is activated by thrombin bound to thrombomodulin (an endothelial glycoprotein). Once activated, protein C inactivates by proteolytic clivage clotting factors Va and VIIIa, exerting an important anticoagulant effect. Its action requires the presence of a cofactor, protein S, which is also a vitamin K dependent liver secreted protein. Only 40% of protein S circulates free in the plasma and acts as a cofactor for protein C, whereas 60% of plasma protein S is bound to c4bBP, a protein which also transports the c4b fraction of the complement.

The physiological importance of the protein C system as an anticoagulant mechanism is demonstrated by the thrombotic events that occur in patients with deficiencies of either protein C or protein S (2, 4,10). Activated protein C and the cofactors of the protein C pathway also exert a more recently revealed cytoprotective (antiinflammatory and antiapoptotic) activity due to direct effects on cells (11).

As surgical procedures are well known for their high thrombotic risk, the behavior of protein C and protein S were investigated in critically ill surgical patients developing an acute phase reaction. Serum cholinesterase activity was also investigated, as a marker of hepatic proteosynthesis.

### MATERIAL AND METHODS

#### *Patients.*

For the protein C study:

*Control subjects.* 32 healthy subjects (13 men, 19 women), aged 20- 60 years

*Critically ill surgical patients.* 29 patients (19 men, 10 women), aged 26-79 years. Blood was collected 5-6 days after major abdominal surgery for mesenteric infarction (5 patients, 2 of them obese), colonic neoplasma (7 patients), gastric carcinoma (2 patients), strangulating

intestinal obstruction (3 patients), acute peritonitis (3 patients), acute pancreatitis (3 patients), severe thoracic and abdominal trauma (6 patients). Only 2 subjects had low platelet count ( $< 100 \times 10^9/l$ ) and low fibrinogen levels ( $< 1,5 \text{ g/l}$ ). All the patients were in a critical clinical condition; 12 of these patients had a lethal outcome in the intensive care unit.

For the protein S study:

*Control subjects.* 10 healthy subjects (4 men., 6 women), aged 27-62 years.

*Critically ill surgical patients.* 12 patients (10 men, 2 women), aged 42-85 years. Blood was collected 5-9 days after major abdominal surgery for colonic neoplasma (1 patient), gastric neoplasma (2 patients), mesenteric infarction (2 patients), hydatid cyst of the liver (1 patient), retroperitoneal tumor (1 patient), acute peritonitis (4 patients). In spite of the relative heterogeneity of the group due to the different etiology, all these subjects were in a clinical critical condition and displayed an acute phase reaction. None of these patients displayed signs of a disseminated intravascular coagulopathy.

**Methods.** Blood was harvested in fasting conditions. Plasma protein C antigen and protein S antigen were measured in platelet poor citrated plasma (PPP). The samples were kept frozen at  $-20^{\circ}\text{C}$  and rapidly thawed at  $37^{\circ}\text{C}$  30 minutes prior to the assay. PC:Ag and PS:Ag were measured using ELISA commercial kits from Asserachrom Diagnostica Stago (France). The kits were provided with their own standards. Serum cholinesterase activity was measured on a Beckman analyser. Fibrinogen was measured colorimetrically.

**Statistics.** Results were expressed as mean  $\pm$  standard error of the mean (SEM). Significance of differences between groups of results was calculated using the Student's t-test.

## RESULTS

As compared to the control group, plasma PC:Ag and serum cholinesterase activity were decreased in the critically ill surgical patients (Table 1). The 12 surgical patients with lethal outcome had lower levels of PC:Ag ( $53,4 \pm 5,66\%$ ) than the 17 survivors ( $70,3 \pm 5,07\%$ ), but the difference was not statistically significant.

Table 1.

Serum cholinesterase (CHE) activity, plasma PC:Ag and plasma fibrinogen level in healthy control subjects and in critically ill surgical patients. Mean  $\pm$  SEM. Significance versus control subjects \* $p < 0,001$

Group	Number of subjects	Che activity(U/l)	Fibrinogen (g/l)	PC activity (% of standard plasma)
Controls	32	$8032 \pm 501$	$2,57 \pm 0,09$	$90,6 \pm 2,8$
Critically ill	29	$3921 \pm 411^*$	$4,29 \pm 0,42^*$	$63,3 \pm 4,21^*$

Plasma PS:Ag was found to be significantly decreased in surgical patients when compared to control subjects (Table 2). Serum cholinesterase activity was also obviously diminished in surgical patients.

Table 2.

Serum cholinesterase (CHE) activity, plasma PS:Ag and plasma fibrinogen level in healthy control subjects and in critically ill surgical patients. Mean  $\pm$  SEM. Significance versus control subjects \* $p < 0,001$

Group	Number of subjects	Che activity (U/l)	Fibrinogen (g/l)	PS activity (% of standard plasma)
Controls	10	7966 $\pm$ 479	2,64 $\pm$ 0,14	80,4 $\pm$ 5,16
Critically ill	12	3650 $\pm$ 535*	5,42 $\pm$ 0,5*	59,23 $\pm$ 4,96*

## DISCUSSION

Decreased levels of plasma protein C and plasma protein S were reported in critically ill surgical patients (5,7), but these findings were interpreted as a consequence of a persistent consumption. No attempt was made to associate this reduction with impaired or dysregulated hepatic synthesis.

In the present studies, the low levels of PC:Ag and PS:Ag, as well as reduced cholinesterase activity in patients without evidence of consumption coagulopathy are more suggestive for an impaired hepatic synthesis, in agreement with Rodeghiero (15). On the other hand, it is also possible that the acute phase reaction developed during the surgical procedure may cause a switch in the hepatic protein synthesis towards the increased production of acute phase proteins (such as fibrinogen), while reducing the secretion of albumin, cholinesterase and presumably of protein C and protein S. Inflammatory mediators such as TNF $\alpha$  can inhibit the protein C anticoagulant pathway, by down-regulating the expression of the protein C activation complex (thrombomodulin and the endothelial cell protein C receptor), and up-regulating  $\alpha$ 1-antitrypsin (an inhibitor of activated protein C) (6).

The clinical relevance of these observations should be interpreted in the context of an overall view, considering the other changes in the hemostasis parameters that occur during surgical procedures, such as antithrombin low levels, high levels of fibrinogen, factor VIII:c, von Willebrand factor (1, 13) and of fibrinolysis inhibitor PAI 1, which all contribute to a prothrombotic status.

A new perspective for the clinical significance of the reduction of protein C and protein S levels is suggested by the recent studies concerning the role of the protein C pathway in inflammation and apoptosis. More recent basic and preclinical research on activated PC has characterized the direct cytoprotective effects of APC that involve gene expression profile alterations, anti-inflammatory and anti-apoptotic activities and endothelial barrier stabilization. These actions generally require endothelial cell protein C receptor (EPCR) and protease activated receptor-1. Because of these direct cytoprotective actions, activated PC reduces mortality in murine endotoxemia and severe sepsis models and provides neuroprotective benefits in murine ischemic stroke models. Furthermore, activated PC reduces mortality in patients with severe sepsis (PROWESS clinical trial)(9,12). According to Gierer (8), activated protein C reduces tissue inflammation, hypoxia and apoptosis in experimentally injured skeletal muscles.

Until recently, the physiologic role of the PS-C4BP complex had remained uncertain. Some years ago, it was suggested that PS could act as a bridge between coagulation and inflammation, by localizing C4BP to negatively charged phospholipids and controlling complement proliferation at sites where the coagulation system was activated (14).

Considering these recent findings one may presume that in surgical patients the low levels of protein C and protein S subsequent to the acute phase reaction may contribute not only to prothrombotic status, but also to the amplification of the inflammatory process.

Because of these pleiotropic properties, activated PC and the protein C pathway components have important roles in both regulation of the coagulation process and inflammatory response, and provide opportunities for therapeutic treatment of complex and challenging medical disorders, including thrombosis, severe sepsis and stroke.

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