Endothelial-related coagulation in cardiac surgery

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Summary
The endothelium appears to play an important role in the regulation of intravascular coagulation. Thrombomodulin is one of the anticoagulant substances that is expressed by endothelial cells. The influence of age and illness on the thrombomodulin–protein C system was studied prospectively in 80 cardiac surgery patients. Patients > 70 yr old (n = 20) were compared with patients < 50 yr (n = 20) (group I), and patients undergoing a simple cardiac procedure (n = 20) were compared with patients scheduled for complex surgery (n = 20) (group II). Thrombomodulin (normal < 40 ng ml⁻¹), protein C and (free) protein S plasma concentrations were measured by enzyme-linked immunosorbent assays (ELISA) after induction of anaesthesia (baseline values), during and after cardiopulmonary bypass (CPB), at the end of surgery, 5 h after CPB and on the first day after operation. Blood loss and use of homologous blood and blood products were significantly greater in patients > 70 yr and in those undergoing complex surgery. At baseline, thrombomodulin concentration was increased in patients undergoing complex surgery (mean 52 (SD 9) ng ml⁻¹). After bypass and after operation, thrombomodulin increased most in patients > 70 yr (from 40 (8) to 78 (10) ng ml⁻¹) and in those patients who underwent complex cardiac operations (from 52 (8) to 79 (10) ng ml⁻¹) (P < 0.05). Changes in protein C and protein S concentrations were similar in all groups. On the first day after operation only, protein C concentrations were reduced in patients > 70 yr and in patients who underwent complex cardiac surgery. Older patients and those who underwent complex cardiac surgery had greater changes in the thrombomodulin–protein C system which may contribute to increased postoperative blood loss. (Br. J. Anaesth. 1995; 75: 174–179)

Key words
Blood, coagulation, Surgery, cardiovascular, Heart, cardiopulmonary bypass, Complications, coagulopathy, Age factors.

Disturbances in coagulation homeostasis are common in patients undergoing cardiac surgery [1]. The imbalance between coagulation activation secondary to the contact of blood with synthetic, non-endothelialized surfaces and reactive fibrinolysis may be important for the bleeding tendency or micro-vascular thrombosis in these patients [2, 3]. Advanced age and critical illness may also affect the coagulation system because of a shift to a procoagulant state [6].

It is increasingly evident that the endothelium is not just a passive barrier between the circulating blood and tissue. The inhibitors of the coagulation system (antithrombin III (AT III) and protein C–protein S) need endothelial factors to counteract procoagulant activity [6, 7]. The endothelium secretes and expresses compounds by which it is involved in the modulation of haemostasis [6, 8, 9]. Thus apart from factor-related and platelet-associated coagulation, the endothelium represents a new dimension in the concept of a balanced haemostatic system. One of the substances that is expressed by the endothelial cell is thrombomodulin [10]. It neutralizes thrombin clotting activity and accelerates thrombin-catalysed activation of protein C to activated protein Ca [11], thus converting thrombin from a procoagulant protease to an anticoagulant [12]. Moreover, the thrombomodulin–thrombin complex and protein Ca, together with protein S, are potent inhibitors of coagulation factors Va and VIIIa [11]. The imbalance between procoagulants and physiological anticoagulants such as thrombomodulin could produce marked disturbances in coagulation [9, 11, 13]. The factors on which changes in the thrombomodulin–protein C system depend are not fully elucidated, particularly in the critically ill and in patients undergoing cardiac surgery. Thus the aim of the present study was to assess the contribution of age and severity of illness to endothelial-related coagulation in cardiac surgery patients.

Patients and methods
We studied 80 patients undergoing cardiac surgery. The study was approved by the hospital’s Human Research Ethics Committee and informed consent was obtained from all patients. Exclusion criteria were any severe preoperative coagulation disorders, and medication with heparin, aspirin or other...
cyclooxygenase inhibitors within the preceding 7 days, recent operations, insulin-dependent diabetes mellitus and severe renal (plasma creatinine concentration > 40 umol litre$^{-1}$) or liver dysfunction. Patients were allocated to two groups: group I (n = 40); patients > 70 yr (n = 20) were compared with patients < 50 yr (n = 20); group II (n = 40); patients undergoing simple cardiac procedures (n = 20) were compared with patients undergoing complex surgery (n = 20). The age of these patients ranged from 55 to 70 yr.

Induction and maintenance of anaesthesia were standardized and comprised weight-related doses of fentanyl, midazolam and pancuronium. No volatile anaesthetic or nitrous oxide was used throughout the study. The lungs were ventilated mechanically with an $F_{\text{io}_{2}}$ of 1.0, and a positive end-expiratory pressure of 5 cm H$_{2}$O was used before and after cardiopulmonary bypass (CPB). The entire anaesthetic was carried out by anaesthetists not involved in the study and blinded to the grouping.

CARDIOPULMONARY BYPASS

Five minutes before the start of CPB, anticoagulation was produced with bovine heparin 300 u. kg$^{-1}$. Anticoagulation was monitored by measuring activated clotting time (ACT, using kaolin with a Hemocheck system (International Technidyne Corp., Edison, NJ, USA)). Additional heparin 150 u. kg$^{-1}$ was given when ACT was < 400 s. CPB was carried out with a capillary oxygenator (Monolyth, Sorin, Turino, Italy). A flow of 2.4 litre min$^{-1}$ m$^{-2}$ was maintained throughout CPB, and moderate hypothermia was used (lowest rectal temperature 33.5 $\pm$ 0.2 $^\circ$C). In all patients high-dose aprotinin was given (aprotinin 2 million iu before CPB, 500 000 iu h$^{-1}$ until the end of operation, 2 million iu added to the prime). The circuit prime consisted of 1000 ml of 5% glucose solution, 1000 ml of Ringer's solution and 250 ml of 5% albumin. When the haemoglobin concentration was < 7 g dl$^{-1}$, packed red blood cells (PRBC) were added to the perfusate. When necessary, Ringer's solution was added to maintain reservoir volume. A mono-atrial cannula was used to return venous blood to the circuit and all fluids (cooling, venting, suction) were drained into the circuit. Within 20 min after the start of CPB, the perfusate was concentrated using an ultrafiltration device (haemofilter HF-80, Fresenius, Bad Homburg, FRG) to maintain haemoglobin concentration at 8–9 g dl$^{-1}$ during the bypass period. During weaning from bypass, as much pump blood as was necessary to keep pulmonary capillary wedge pressure > 10 mm Hg but < 14 mm Hg was infused. After termination of CPB, the residual blood of the extracorporeal oxygenation equipment was concentrated using the HF-80 system, and the autologous blood was retransfused. Protamine sulphate was given to antagonize heparin in a 1:1 ratio with the initial dose of heparin. Additional protamine was administered if ACT was > 150 s.

Hemoglobin, platelet count, ACT, fibrinogen and AT III plasma concentration, and partial thromboplastin time (PTT) were measured in arterial blood samples. Plasma concentrations of thrombomodulin were measured by enzyme-linked immunosorbent assay (ELISA) using a polyclonal antibody against human thrombomodulin (Diaagnostica Stago, Asnieres Cedex, France) [14]. The ELISA uses two monoclonal antibodies which react at different sites of the thrombomodulin molecule and do not interfere with each other's binding. Normal values of thrombomodulin in healthy volunteers assessed by this method were reported to be < 30–40 ng ml$^{-1}$ [14]. Protein C and protein S were measured by commercial ELISA kits (Boehringer-Mannheim, Mannheim, Germany) [15]. Protein S was quantified as free protein S after bound protein S was removed from plasma by precipitation with polyethylene. All ELISA results represent the means from duplicate measurements. All measurements were carried out after induction of anaesthesia (baseline values), 20 min after onset of CPB (after haemodilution by haemofiltration), after separation from CPB (before injection of protamine), at the end of surgery, 5 h after the end of CPB and on the morning of the first day after operation. Volume replacement, urine output and blood loss from post-bypass suction and chest tube drainage were also documented. PRBC were given when the haemoglobin concentration was < 9 g dl$^{-1}$. Fresh frozen plasma (FFP) was given when bleeding exceeded 200 ml for 3 h, platelet count was > 50 x 10$^9$ litre$^{-1}$ and ACT was > 200 s. All volume therapy and inotropic support (adrenaline, noradrenaline) were given by physicians who were not involved in the study and who were blinded to the grouping.

STATISTICAL ANALYSIS

Data are expressed as mean (SD). One- and two-factorial analyses of variance (ANOVA) followed by Scheffe tests were used for statistical interpretation. The chi-square test was used to analyse differences for homologous blood. $P < 0.05$ was considered significant.

Results

Details of patients and surgery are shown in table 1. Duration of CPB was significantly longer in patients undergoing complex cardiac procedures. Blood loss after bypass and after operation was greatest in patients > 70 yr and in the group undergoing complex surgery ($P < 0.05$). The use of homologous blood and blood products (FFP) was also greatest in these two groups. The degree of haemodilution was similar in both groups (table 2). AT III and fibrinogen plasma concentrations (table 2) did not change throughout the study. PTT was longer at baseline in patients undergoing complex surgery, but did not differ in the postoperative period.

Plasma concentrations of thrombomodulin (fig. 1) at baseline were significantly greater in patients undergoing complex cardiac surgery (52 (9) ng ml$^{-1}$). After bypass and after operation, concentrations of thrombomodulin increased most in patients > 70 yr (from 41 (8) to 78 (10) ng ml$^{-1}$) and in patients who...
underwent complex procedures (from 52 (8) to 79 (10) ng ml⁻¹). In patients < 50 yr, plasma concentrations of thrombomodulin remained almost unchanged compared with baseline values, but increased slightly in patients who underwent simple procedures. On the first day after operation, plasma concentrations of thrombomodulin were still increased in patients > 70 yr (78 (9) ng ml⁻¹) and in patients who underwent complex surgery (80 (10) ng ml⁻¹) and were significantly greater than in the two other subgroups.

Protein C plasma concentrations (fig. 2) did not vary from normal baseline values in any group except on the first day after operation. In patients > 70 yr and in those who underwent complex procedures, protein C concentrations were reduced
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Discussion

There is a large body of literature showing that cardiac surgery using CPB is associated with coagulation abnormalities [1,2]. The pathogenesis of these alterations is complex and includes platelet defects, (hyper-)fibrinolysis and changes in plasma coagulation factors. Recently it has become clear that the endothelium is involved in the regulation of coagulation. The resistance to clot formation of the endothelial surface is mediated by various mechanisms, including production of substances such as prostacyclin (PGI₂), tissue plasminogen activator (tPA) or heparin-like molecules [6,7,10]. Thrombomodulin, another substance produced by the endothelium, appears to play a central role in this endothelial-related regulation of haemostasis [7].

The reduction of thrombomodulin expression on the endothelial cell surface may be important for the development of coagulation disorders in conditions such as cancer, disseminated intravascular coagulation (DIC), inflammation and sepsis [8,11]. Inflammatory cytokines are known to be released by CPB [16]. These mediators are reported to shift the haemostatic balance of the surface of the endothelial cells towards prothrombosis, most likely because of down-regulating thrombomodulin [17]. Thus by reducing thrombomodulin expression, these mediators influence thrombomodulin-thrombin-mediated protein C activation and thus inhibit an important pathway of physiological anticoagulation. Continuous activation of coagulation will finally result in a hypocoagulable state, which is complicated by reactive hyperfibrinolysis.

One problem with the investigation of endothelial-related coagulation is that some elements are expressed on the endothelial surface and are (normally) only minimally released into the circulating blood [12]. Thrombomodulin is mainly bound to endothelial cell surfaces but is also present in a soluble form in the circulation [18]. Soluble thrombomodulin appears to be a truncated (by proteolytic cleavage) form of membrane-bound, endothelial surface thrombomodulin [19] but still possesses potent protein C activating properties [18,19]. Because this circulating, non-membrane-bound thrombomodulin is increased in severe illness, particularly in situations with generalized inflammatory response, plasma thrombomodulin is thought to be a marker of endothelial cell damage [20,21].

In the present study, plasma concentrations of thrombomodulin after bypass were higher in patients who underwent complex cardiac surgery. The longer
CPB time may be important as it is known that endotoxin, some cytokins and tumour necrosis factor (TNF) concentrations are greater after prolonged CPB [16,22]. Moreover, thrombin is generated during CPB in spite of adequate anticoagulation with heparin. All of these factors are reported to be important stimulants for increased expression, liberation, or both, of thrombomodulin from endothelial cells into circulating blood [7,9,11,23]. Furthermore, it can be assumed that patients undergoing complex surgery were more ill than those undergoing simple procedures. Interestingly, the former patients had increased plasma concentrations of thrombomodulin before operation. Thus other factors may also have contributed: hypoxia and microcirculatory abnormalities [24], release of proteinases from activated white cells [25], activation of the complemet system [26] and activation of platelets.

Age and duration of CPB appear to be important contributors to changes in thrombomodulin as plasma concentrations were significantly higher in patients > 70 yr. Thus it can be assumed that the older patients were more susceptible to CPB-related endothelial damage with consecutive increase in plasma thrombomodulin.

Changes in protein C and (free) protein S plasma concentrations did not parallel those of thrombomodulin. The decrease during CPB is most likely caused by haemodilution and increased coagulation activation and elimination by the reticuloendothelial system [27]. The lesser protein C plasma concentrations on the first day after operation in our patients > 70 yr and in patients who underwent complex cardiac surgery may also reflect activation and consumption of the thrombomodulin–protein C system in response to generated thrombin. Knobl and co-workers [28] reported an increase in protein S soon after beginning CPB. Protein C, however, decreased significantly during CPB and remained reduced after bypass, which may contribute to the well known bleeding tendency of cardiac surgery patients [28]. Thrombomodulin, however, plays a central role in the anticoagulant properties of protein C. The rate of conversion of protein C to (active) protein Ca by thrombin is slow but the presence of thrombomodulin markedly increases the rate of protein C activation by thrombin (about 20000 times) [10,12]. Thus the rapid clearance of thrombin from the circulation could also be ascribed to thrombomodulin, which functions as a high-affinity, active site, independent receptor for thrombin on the endothelium. Soluble circulating thrombomodulin may contribute also [29].

Changes in standard coagulation variables (AT III, fibrinogen, PTT) were similar in all patients. ACT was always > 400 s and PTT > 250 s, which is thought to be a safe degree of anticoagulation during CPB. There are only a few studies on endothelial-related coagulation in cardiac surgery. In patients undergoing hypothermic CPB, Tanaka and colleagues [30] found a significant decrease in thrombomodulin, protein C and protein S during bypass. One hour after CPB, thrombomodulin had exceeded baseline values. Confounding factors are that both bubble and membrane oxygenators were used and mean temperature during CPB was 24.8 °C. Both factors may markedly contribute to changes in coagulation.

High-dose aprotinin was used in all patients in the present study. It is given routinely in our department to patients undergoing complex procedures. Thus to eliminate a possible aprotinin-induced differences in endothelial-related coagulation in cardiac surgery. In patients undergoing hypothermic CPB, Tanaka and colleagues [30] found a significant decrease in thrombomodulin, protein C and protein S during bypass. One hour after CPB, thrombomodulin had exceeded baseline values. Confounding factors are that both bubble and membrane oxygenators were used and mean temperature during CPB was 24.8 °C. Both factors may markedly contribute to changes in coagulation.

Blood loss and use of homologous blood in the present study were greater in patients > 70 yr and in patients who underwent complex cardiac surgery. Although this study is based on a rather small patient population, it can be assumed that the changes in the thrombomodulin–protein C system may have contributed to the blood loss. It was, however, not the major aim of the present study to correlate changes in the thrombomodulin–protein C system with blood loss or need for homologous blood. For this purpose a larger patient population is required.

References
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![Figure 3](https://academic.oup.com/bja/article-abstract/74/2/174/452348/178)
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