Anesthesiology 2001; 95:1169–74

The Effects of Aprotinin on Thromboelastography with Three Different Activators

Michael S. Avidan, F.C.A.S.A., Jorge Da Fonseca, F.R.C.A.,† Kiran Parmar, A.I.M.L.S.,‡ Emma Alcock, F.R.C.A.,§ José Ponte, Ph.D., F.R.C.A.,‖ Beverley J. Hunt, M.D., F.R.C.P., F.R.C.Path.#*

Background: **Thromboelastography is used for assessment of hemostasis. Adherence to thromboelastography-guided algorithms and aprotinin administration each decrease bleeding and blood product usage after cardiac surgery. Aprotinin, through inhibition of kallikrein, causes prolongation of the celite-activated clotting time and the activated partial thromboplastin ratio. The aim of this study was to assess the effects of aprotinin on the thromboelastography trace.**

Methods: **Three activators were used in the thromboelastography: celite (which is widely established), kaolin, and tissue factor. Assessment was performed on blood from volunteers and from patients before and after cardiac surgery.**

Results: **The tissue factor–activated thromboelastography trace was unaffected by the addition of aprotinin. When celite and kaolin were used as activators in the presence of aprotinin, the reaction time (time to clot formation) of the thromboelastography trace was prolonged ($P < 0.0001$) and the maximum amplitude (clot strength) was decreased ($P < 0.05$). With celite as an activator, the addition of aprotinin decreased ($P < 0.05$) the thromboelastography α angle (rate of clot extension). The reaction time of the celite-activated trace correlated with the activated partial thromboplastin ratio ($P < 0.01$). The reaction time of the tissue factor–activated trace correlated with the international normalized ratio ($P < 0.01$).**

Conclusion: **The thromboelastography trace is altered in the presence of aprotinin when celite and kaolin are used as activators but not when tissue factor is the activator.**

BLEEDING is a significant complication of major surgery.[1] Clinical trials using high-dose aprotinin have shown reductions of blood loss by 30% in open cardiac surgery.[2] Aprotinin is a Kunitz-type serine protease inhibitor with greatest affinity for trypsin and plasmin.[2] At high dosage (plasma concentration of approximately 200 KIU/ml), aprotinin also inhibits kallikrein and thus the intrinsic pathway of coagulation.[2] The current opinion is that aprotinin reduces bleeding mainly through plasmin inhibition.[3]

During cardiopulmonary bypass (CPB), heparin prevents clotting in the extracorporeal circuit. Heparinization during cardiac surgery is usually monitored by the activated clotting time (ACT). When aprotinin is administered, the celite-activated ACT is prolonged.[4] This prolongation is less pronounced when kaolin is used as the activator for the ACT.[5–7] A similar effect is also seen in laboratory assays of the intrinsic pathway, such as the activated partial thromboplastin ratio (APTR).[8] This prolongation is variable depending on the activating agent used. Celite and kaolin both act as contact surfaces for the intrinsic coagulation pathway and can be used as activators in the APTR.

Thromboelastography provides a global assessment of hemostasis giving sequential information on the time to initial clot formation, the clot strength, and the degree of fibrinolysis.[9] Studies using thromboelastography during cardiac surgery have shown a decrease in perioperative blood loss and reduced transfusion of blood products.[10,11]

Because the "native" thromboelastography trace is slow to provide information, the addition of activators has been proposed to reduce the time to trace generation. Celite increases the rapidity with which results become available and improves test reproducibility.[12] The use of kaolin for thromboelastography has not been described. Recombinant tissue factor (TF) has recently been suggested as an activator for thromboelastography[13] and may provide a more accurate model of coagulation because it is the TF pathway (extrinsic coagulation pathway), rather than contact activation (intrinsic coagulation pathway), that provides the major impetus for clot formation during CPB.[14]

Aprotinin prolongs the reaction time (R) of the "native" thromboelastography trace.[15,16] This study was designed to assess the nonfibrinolytic effects of aprotinin on the trace in the presence of three activators (celite, kaolin, and TF), and to compare thromboelastography parameters with laboratory tests of coagulation.

Materials and Methods

Approval for the study was obtained from the King's College Hospital Ethics Committee (London, United Kingdom).

Details of Volunteers and Patients

Blood samples were drawn from 45 healthy volunteers and 36 adult patients undergoing elective cardiac sur-

* Assistant Professor, † Consultant, § Clinical Lecturer, ‖ Senior Lecturer, Department of Anesthesiology, ‡ Research Scientist, # Consultant and Honorary Senior Lecturer, Department of Haematology.

Received from the Departments of Anaesthesia and Haematology, Guy's, King's, and St. Thomas' Medical School, London, United Kingdom. Submitted for publication November 20, 2000. Accepted for publication June 8, 2001. Supported by The Royal College of Anaesthetists through the British Journal of Anaesthesia Small Project Grant, Oxford, United Kingdom, and the National Blood Services, London, United Kingdom. Medicell U.K., London, United Kingdom, provided thromboelastography consumables at discounted prices and lent the investigators a thromboelastography machine. Additional funds were raised by the Academic Department of Anaesthesia, King's College London, London, United Kingdom. Presented in part at the Annual Spring Meeting of the Association of Cardiothoracic Anaesthetists, Cambridge, United Kingdom, April 16, 1999.

Address reprint requests to Dr. Avidan: Department of Anesthesiology, Washington University School of Medicine, Campus Box 8054, 660 South Euclid Ave., St. Louis, Missouri 63110-1093. Address electronic mail to: avidanm@msnotes.wustl.edu.

gery at King's College Hospital. Volunteers were doctors and nurses who agreed to donate blood specimens for this study. Patients gave verbal consent for thromboelastography tests to be performed on their blood at the same time as blood was drawn for other routine blood tests. Patients with known coagulopathies, those taking anticoagulants, and those with liver or renal dysfunction were excluded from the study.

Blood Sampling

Healthy Volunteers. Blood was drawn into tubes containing sodium citrate (0.105 M; ratio, 9:1). After thromboelastography tests had been performed, the blood was centrifuged at 2,000*g* to obtain platelet-poor plasma. Samples were stored in aliquots at −70°C until the time of assay.

Cardiac Surgery Patients Receiving Aprotinin. Blood was drawn from 26 patients who received aprotinin as part of their routine management. Blood samples were drawn from a free-flowing, 14-gauge central catheter immediately before and 5 min after aprotinin administration. This was before surgery and heparin administration. Thromboelastography tests were performed using fresh blood.

Cardiac Surgery Patients after CPB. Blood was drawn from 10 patients into a tube containing sodium citrate 1 h after protamine administration after CPB. These patients had not received aprotinin.

Laboratory International Normalized Ratio, APTR, and Fibrinogen

The international normalized ratio (INR), APTR, and fibrinogen values were obtained using a nephelometric method on an ACL 300R® machine (Instrumentation Laboratory, Warrington, Cheshire, United Kingdom).

Preparation of Thromboelastography Cuvettes

Celite and kaolin were obtained in powder form. A solution of 1 g/100 ml (1%) was mixed using normal saline to ensure that there was sufficient quantity for the whole study. The solutions were stored in a refrigerator at 2–8°C in 1.5-ml plastic containers with an aliquot of 37 μl kaolin or celite. At the time of testing, the plastic containers were warmed to room temperature, and 1 ml blood was added, giving final celite or kaolin concentrations of 0.357 mg/ml blood. This is the celite concentration recommended by the manufacturer. There are no previous reports of kaolin used as an activator for thromboelastography; initial dose–response studies (data not shown) suggested that this concentration produced a significant shortening of the R time compared with the "native" trace but retained sensitivity.

Recombinant TF has been used as an activator for thromboelastography in a previous study.[13] Recombinant TF (Innovin®; Dade Behring, Atterbury, Milton Keynes, United Kingdom) was diluted to a concentration of 1:120. Thirty-seven microliters of this solution was decanted into 1.5-ml plastic containers, which were stored at −20°C and warmed to room temperature before the thromboelastography test. One milliliter of blood was added to the containers to yield an equivalent final TF concentration of 0.3 μl/ml blood.

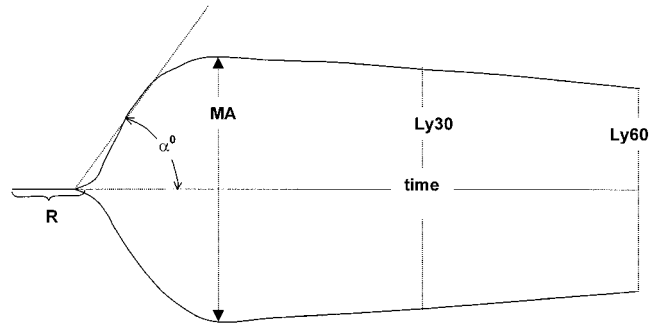

Fig. 1. The typical thromboelastography trace. R = reaction time; α^{o} = α angle; MA = maximum amplitude; Ly30 and Ly60 = percentage lysis at 30 and 60 min, respectively, after MA.

Performance of the Thromboelastography Tests

Two dual-channel thromboelastography machines (TEG®; Haemoscope, Niles, IL) were used in parallel, connected to a notebook computer. The machines underwent regular quality control and calibration. All thromboelastography measurements were performed with the machines prewarmed to 37°C. The parameters recorded included R time, α angle, maximum amplitude (MA), and lysis index at 30 min (percentage reduction of MA at 30 min after the MA). R represents the time to initial clot formation, α angle relates to rate of clot extension, MA relates to clot strength, and the lysis indices reflect the extent of fibrinolysis present (fig. 1).

Sample Processing

Healthy Volunteers. Citrated blood samples were analyzed 60 min after venepuncture. One milliliter sodium-citrated blood was added to a container holding 37 μl of activator (celite, kaolin, or TF). This was mixed, and then 340 μl was transferred into the thromboelastography cuvette to which 20 μl calcium chloride, 0.1 M, had been added.

Cardiac Surgery Patients Receiving Aprotinin. Fresh blood was placed into a sterile plastic container. After 4 min, 1 ml blood was added to a container holding 37 μl of activator (celite, kaolin, or recombinant TF). This was mixed, and then 360 μl was transferred into the thromboelastography cuvette.

Cardiac Surgery Patients after CPB. Thromboelastography tests were performed as described for healthy volunteers between 2 and 5 h after the blood was drawn.

Effects of Aprotinin on Thromboelastography Tests with Volunteers' Blood

Samples from 19 volunteers were run in duplicate with kaolin in two thromboelastography channels and celite

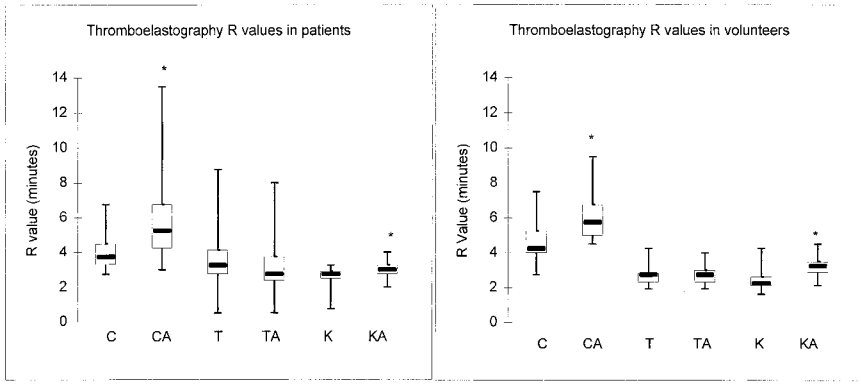

Fig. 2. Box and Whisker plots showing thromboelastography reaction times (R) from tests on blood taken from cardiac surgery patients and healthy volunteers before and after the addition of aprotinin to the blood. Plots show R times using different activators for thromboelastography. C = celite; CA = celite after aprotinin; T = tissue factor; TA = tissue factor after aprotinin; K = kaolin; KA = kaolin after aprotinin. Boxes show median values with interquartile ranges, and whiskers represent the full ranges. *P < 0.01 compared with preaprotinin.

in the other channels. Samples from 18 volunteers were run in duplicate with celite and TF as activators in two channels each. Aprotinin was added to blood samples to obtain a concentration of 200 KIU/ml. This blood was run in the third and fourth channels in each experiment. Thus, with each activator, there was a paired test with and without aprotinin.

Effects of Aprotinin on Thromboelastography Tests with Patients' Blood

Blood was drawn before and after aprotinin administration. Paired celite- and kaolin-activated thromboelastography tests were performed on samples from the first 11 patients, and celite- and TF-activated thromboelastography tests were performed on blood from the subsequent 15 patients.

Dose Response to Aprotinin

Thromboelastography tests were performed using blood from eight volunteers with celite and TF as activators. Three channels were used for each test with aprotinin blood concentrations of 0, 100, and 200 KIU/ml, respectively, in each channel.

Aprotinin after CPB

Blood from 10 patients after surgery was tested with the same methodology described in the previous paragraph.

Statistical Analysis

The Wilcoxon signed rank test was used for paired comparisons. Results are expressed as median difference (95% confidence interval [CI]) and P value for difference. Pearson and Spearman correlations were used where appropriate to evaluate association among parameters.

Table 1. The Effects of Aprotinin on Thromboelastography R Values

Group (N)	Activator	A-0	A-100	A-200
Vol (37)	C	4.3 (4–5.3) [2.5–7.5]	—	5.8* (5–6.8) [3.8–9.5]
Vol (19)	K	2.3 (2.1–2.6) [1.5–4.3]	—	3.3* (2.9–3.5) [1.8–4.5]
Vol (18)	T	2.8 (2.3–2.8) [1.5–4.3]	—	2.8 (2.3–3) [1.5–4]
Pat (26)	C	3.8 (3.3–4.5) [2.8–6.8]	—	5.3* (4.3–6.8) [3–13.5]
Pat (11)	K	2.8 (2.5–2.9) [0.8–3.2]	—	3* (2.8–3.3) [2–4]
Pat (15)	T	3.3 (2.8–4.1) [0.5–8.8]	—	2.8 (2.4–3.8) [0.5–8]
Vol (8)	C	7.3 (6.2–7.7) [5.8–8.3]	8.1* (7.8–8.9) [7.1–10.3]	8.1* (7.1–10.3) [6.8–13.5]
Vol (8)	T	2.9 (2.8–3.8) [1.9–4.3]	3.1 (2.8–3.8) [1.8–4.5]	3 (2.8–3.8) [1.5–4]
Pat post CPB (10)	C	6.1 (4.4–9.9) [3.3–13.8]	7.5* (4.9–14.1) [3.8–16.3]	7.4* (4.8–14.5) [4–17]
Pat post CPB (10)	T	4.5 (3.5–8.6) [2.8–13.3]	4.9 (3.3–11.4) [2.5–17.8]	4.3 (3.2–11.6) [2.3–16.5]

The first column shows the group (volunteers or patients) and the number. The second column is the activator; celite (C), kaolin (K), or tissue factor (T). The next three columns reflect increasing aprotinin concentrations; A-0 = 0 KIU/ml; A-100 = 100 KIU/ml; A-200 = 200 KIU/ml. Results are shown as median values in minutes with interquartile ranges and full ranges in brackets.

* $P < 0.05$ compared with preaprotinin value.

Vol = volunteers; Pat = patients; Pat post CPB = patients post-cardiopulmonary bypass.

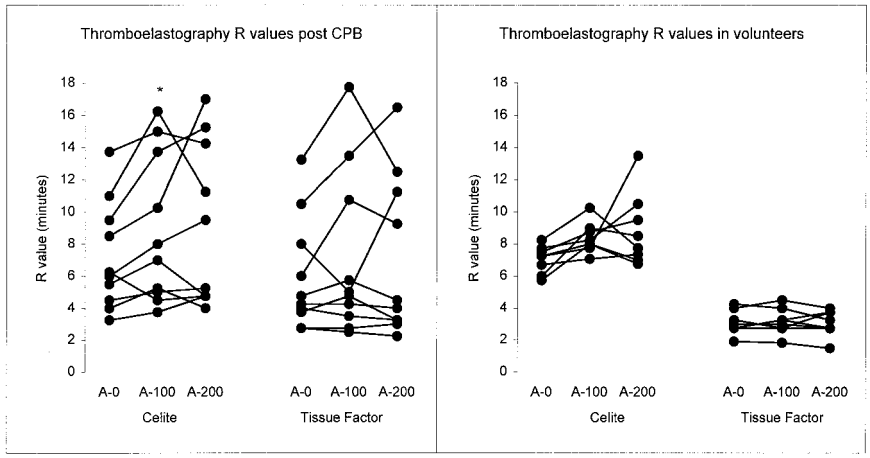

Fig. 3. The effects of increasing blood concentrations of aprotinin on thromboelastography reaction time (R) with celite and tissue factor as activators. A-0 = no aprotinin; A-100 = 100 KIU/ml aprotinin; A-200 = 200 KIU/ml aprotinin. *$P < 0.05$ (Friedman analysis of variance).

Results are expressed as correlation coefficient (95% CI) and *P* value for correlation). The Friedman test was used for analysis of variance. For agreement between tests, Altman and Bland bias plots and Passing-Bablok method comparisons were used. Bias with 95% CIs are reported. Data distribution was evaluated with the Shapiro-Wilk normality test. Statistical analysis was performed using Analyse-It® Statistical Software (Analyse-It, Leeds, United Kingdom).

Results

The Effects of the Activators on the Thromboelastography Trace

The thromboelastography R values were shorter when both kaolin and TF were used as activators compared with celite activation ($P < 0.001$). There was a strong correlation between kaolin- and celite-activated R values (r = 0.58 [CI = 0.27–0.78], $P = 0.001$) but not between celite- and TF-activated R values. Bias of 1.8 min (CI = 1.4–4.1) was detected between R values with celite and kaolin as activators. There was a small but consistent increase in the α angle with kaolin compared with celite (bias = 2° [CI = 1–3]) with a strong correlation between these values (r = 0.84 [CI = 0.68–0.92], $P < 0.001$). There was no significant difference in the celite and TF α angles, but there was also no correlation between these values. Similarly, there was a small increase in the MA with kaolin compared with celite (bias = 5 mm [CI = 2.3–7.6], $P < 0.001$) with a strong correlation between these values (r = 0.89 [CI = 0.79–0.95]). There was no significant difference or bias between the TF and celite MAs, and, unlike the R values and α angles, these values were strongly correlated (r = 0.79 [CI = 0.61–0.89], $P < 0.001$).

The Effects of Aprotinin

Aprotinin prolonged the R time of the thromboelastography trace ($P < 0.001$) when celite and kaolin were used as activators but not when TF was the activator (fig. 2 and table 1) with both volunteers' and patients' blood. There remained a strong correlation between the celite and kaolin R values after aprotinin (r = 0.67 [CI = 0.4–0.83], $P < 0.001$). The celite and TF R values did not correlate after aprotinin. There was also a decrease in the celite α angles (median decrease = −2.5° [CI = −1.8 to −3.3], $P < 0.001$) and MAs (median decrease = −2 mm [CI = −1.3 to −3], $P < 0.001$) after aprotinin. The kaolin α angles did not decrease significantly, but the kaolin MAs were decreased (median decrease = −1.3 mm [CI = 0 to −2.5], $P = 0.03$) after aprotinin. TF α angles and MAs, like TF R times, were not significantly affected by aprotinin. There was a consistent prolongation of the APTR after aprotinin (median increase = 0.65 [CI = 0.6–0.7], $P < 0.001$). Fibrinogen concentrations and the INR were unaffected by aprotinin.

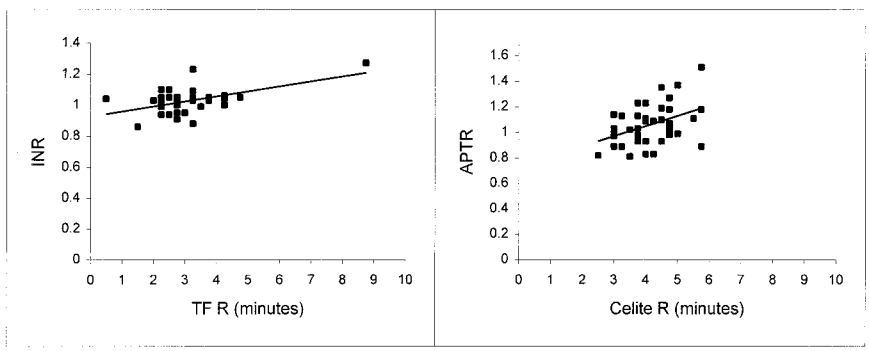

Fig. 4. Graphs showing the correlation between the international normalized ratio (INR) and the tissue factor (TF)–activated thromboelastography reaction time (R; *left*) and the activated partial thromboplastin ratio (APTR) and the celite-activated thromboelastography R time (*right*).

The aprotinin dose–response curves on blood tested from volunteers and from patients after CPB showed that at blood aprotinin concentrations of both 100 and 200 KIU/ml, aprotinin prolonged the R times of celite-activated thromboelastography ($P < 0.05$) but not TF-activated thromboelastography (fig. 3 and table 1).

Comparison of Conventional Clotting Tests with Thromboelastography Parameters

The celite-activated thromboelastography R values correlated with the APTR results (fig. 4) at baseline (r = 0.41 [CI = 0.12–0.64], $P = 0.008$) and after aprotinin (r = 0.53 [CI = 0.29–0.73], $P < 0.001$), but there was no correlation with the INR. The INR was not affected by aprotinin and correlated with the TF-activated thromboelastography R values (fig. 4) both before (r = 0.52 [CI = 0.2–0.74], $P = 0.003$) and after aprotinin administration (r =0.44 [CI = 0.1–0.68], $P = 0.01$). There was no correlation between the TF-activated thromboelastography R values and the APTR. There was a correlation between fibrinogen concentrations and celite α angles (r = 0.39 [CI = 0.09–0.62], $P = 0.01$) as well as celite MA (r = 0.8 [CI = 0.66–0.89], $P < 0.001$). Fibrinogen also correlated with TF MA (r = 0.78 [CI = 0.59–0.89], $P < 0.001$). The platelet count correlated with the celite MA (r = 0.67 [CI = 0.36–0.85], $P < 0.001$) and the TF MA (r = 0.75 [CI = 0.31–0.93], $P < 0.001$).

Discussion

If thromboelastography is to be used with an algorithm to guide the use of blood components during surgery, then it is important to know whether pharmaceutical agents alter its trace. The results of this study show that when celite and kaolin are used as activators for thromboelastography in the presence of aprotinin, there is a prolongation of R time, a decrease in α angle, and a decrease in MA. Therefore, celite and kaolin may not be ideal activators for use in a thromboelastography-guided algorithm, in which prolongation of the R time is a trigger for fresh frozen plasma transfusion and decrease in MA is a trigger for platelet transfusion,[11] in aprotinin-treated patients. TF is a cell surface glycoprotein that serves as the cellular receptor for factor VII, and the TF-FVII complex is a trigger of coagulation *in vivo*.[17,18] Although TF has been described as an activator for thromboelastography,[11,13] the effects of aprotinin have not previously been evaluated in this context. This study suggests that TF is a reproducible activator for thromboelastography, and the trace produced is not affected by aprotinin. This might favor its use in situations in which aprotinin is administered, so that an underlying coagulopathy is not obscured by aprotinin.

Aprotinin is well-recognized to cause prolongation of the ACT and APTR. These assays are specifically designed to evaluate the intrinsic pathway through activation of factor XII, which activates prekallikrein to kallikrein, the latter operating a positive feedback loop in factor XII activation. Aprotinin, as an antikallikrein agent, slows generation of activated factor XII and results in prolonged clotting times in assays of the intrinsic pathway. The prolongation of the celite and kaolin (both factor XII activators) thromboelastography R times by aprotinin is consistent with our understanding, as is the correlation between the APTR and the celite-activated R times. Similarly, the failure of aprotinin to affect TF-activated traces is predictable because aprotinin has no effect on either TF or the subsequently activated extrinsic pathway. As expected, the TF-activated thromboelastography R times correlated with the INR.

Actions of celite and TF at different sites in the coagulation pathways produced differences in the thromboelastography traces when the same blood samples were tested. This could have important clinical applications. For example, TF-activated thromboelastography is more reliable in detecting a warfarin effect than is celite-activated thromboelastography.[19] The relation between thromboelastography parameters and established laboratory tests of coagulation requires in-depth investigation and validation for thromboelastography to gain widespread clinical acceptance.[20,21]

In conclusion, this study showed that aprotinin resulted in several changes in the thromboelastography trace when celite and kaolin were used as activators but not when TF was used. This mirrors the effects of aprotinin on laboratory tests of coagulation and is consistent with the mode of action of aprotinin. The clinical implication is that prolongation of the R time, decrease in the α angle, and decrease in the MA of both celite- and kaolin-activated thromboelastography should be interpreted with caution in the presence of aprotinin.

References

1. Carson JL, Poses RM, Spence RK, Bonavita G: Severity of anaemia and operative mortality and morbidity. Lancet 1988; 1:727–9

2. Davis R, Whittington R: Aprotinin: A review of its pharmacology and therapeutic efficacy in reducing blood loss associated with cardiac surgery. Drugs 1995; 49:954–83

3. Segal H, Hunt BJ: Aprotinin: pharmacological reduction of perioperative bleeding. Lancet 2000; 355:1289–90

4. Hunt BJ, Segal H, Yacoub M: Aprotinin and heparin monitoring during cardiopulmonary bypass. Circulation 1992; 86:410–12

5. Dietrich W, Jochum M: Effect of celite and kaolin on activated clotting time in the presence of aprotinin: Activated clotting time is reduced by binding of aprotinin to kaolin. J Thorac Cardiovasc Surg 1995; 109:177–8

6. Wendel HP, Heller W, Gallimore MJ, Bantel H, Müller-Beissenhirtz H, Hoffmeister HE: The prolonged activated clotting time (ACT) with aprotinin depends on the type of activator used for measurement. Blood Coagul Fibrinolysis 1993; 4:41–5

7. Feindt P, Seyfert UT, Volkmer I, Straub U, Gams E: Celite and kaolin produce differing activated clotting times during cardiopulmonary bypass under aprotinin therapy. Thorac Cardiovasc Surg 1994; 42:218–21

8. Despotis GJ, Alsoufiev A, Goodnough LT, Lappas DG: Aprotinin prolongs whole blood activated partial thromboplastin ratio but not whole blood prothrombin time in patients undergoing cardiac surgery. Anesth Analg 1995; 81: 919–24

9. Mallet SV, Cox DJA: Thromboelastography. Br J Anaesth 1992; 69:307–13

10. Royston D, Von Kier S: Reduced haemostatic factor transfusion using heparinase-modified thromboelastography during cardiopulmonary bypass. Br J Anaesth 2001; 86: 575–8

11. Shore-Lesserson L, Manspeizer HE, De Perio M, Francis S, Vela-Cantos F, Ergin MA: Thromboelastography-guided transfusion algorithm reduces transfusions in complex cardiac surgery. Anesth Analg 1999; 88:312–9

12. Yamakage M, Tsujiguchi N, Kohro S, Tsuchida H, Namiki A: The usefulness of celite-activated thromboelastography for evaluation of fibrinolysis. Can J Anaesth 1998; 45:993–6

13. Khurana S, Mattson JC, Westley S, O'Neill WW, Timmis GC, Safian RD: Monitoring platelet glycoprotein IIb/IIIa-fibrin interaction with tissue factor-activated thromboelastography. J Lab Clin Med 1997; 130:401–11

14. Boisclair MD, Lane DA, Philippou H, Esnouf MP, Sheikh S, Hunt B, Smith KJ: Mechanisms of thrombin generation during surgery and cardiopulmonary bypass. Blood 1993; 82:3350–7

15. De Hert SG, Farooqi NU, Delrue GL, Broecke PW, Vermeyen KM, Adriaensen HF: Dose dependent effect of aprotinin on rate of clot formation. Eur J Anaesth 1996; 13:463–7

16. Chalkiadis GA, Gibbs NM: The effect of aprotinin on thrombelastography in vitro. Anaesth Intensive Care 1996; 24:552–4

17. Rock G, Wells P: New concepts in coagulation. Crit Rev Clin Lab Sci 1997; 34:475–501

18. Rao LV, Pendurthi UR: Tissue factor on cells. Blood Coagul Fibrinolysis 1998; 1:S27–S35

19. Avidan MS, Alcock E, da Fonseca J, Donaldson N, Parmar K, Hunt BJ: Thromboelastography in warfarinized patients. Br J Anaesth 2000; 85:640P

20. Whitten CW, Greilich PE: Thromboelastography: Past, present, and future. ANESTHESIOLOGY 2000; 92:1223–5

21. Samama CM: Thromboelastography: The next step. Anesth Analg 2001; 92:563–4