

Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Fergusson DA, Hébert PC, Mazer CD, et al. A comparison of aprotinin and lysine analogues in high-risk cardiac surgery. *N Engl J Med* 2008;358:2319-31. DOI: 10.1056/NEJMoa0802395.

Appendix A. Description of the study population

Study Population

In this trial, we aimed to study cardiac surgical patients at high-risk of massive postoperative bleeding (exceeding 5%), who were exposed to allogeneic blood products (exceeding 50%) and at increased risk of all adverse clinical events (exceeding 20%). We have chosen high-risk cardiac surgery patients because:

- The high combined event rate would enable us to detect a difference between therapies;
- Aprotinin may save lives and decrease morbidity in high-risk cardiac surgery patients;
- Very high rates of major bleeding and exposure to blood products ensure that the question is relevant;
- Antifibrinolytics have convincingly been shown to decrease exposure to RBCs and blood loss in low-risk procedures; and,
- Any result in high-risk cardiac surgical procedures, including a null result, would be meaningful.

Patient Screening and Recruitment

Cardiac surgery patients meeting the criteria for inclusion into the study were identified pre-operatively in pre-operative and pre-admission clinics and units. The Research Coordinator was introduced to prospective study patients by the cardiac anesthesiologist or surgeon. The Research Coordinator and/or the cardiac surgeon or anesthesiologist explained the study to the patient and provided a copy of the study letter of information. Any questions the patients had at the time were answered.

The patients were asked if they were interested in participating in the study. If a patient's answer was yes, he/she was required to sign a study consent form. The patient retained a copy of the study letter of information. The signed consent form was maintained in the patient's study chart. Once consent was obtained, the Research Coordinator provided the Research Pharmacist with the patient's name and hospital identification number, the patient's weight, the date and type of surgery, and attending surgeon and anesthesiologist.

The Research Coordinator at each centre also maintained a record of all patients screened for inclusion in the study and transmitted screening information to the Coordinating Centre on a weekly basis. For patients who did not wish to participate in the study, or who met one or more of the exclusion criteria, the Research Coordinator only provided to the Coordinating Centre the patient's age, gender, reason for study exclusion and whether or not the patient was discharged dead or alive. The Research Coordinator did not include patient-specific information on the screening log (i.e., Hospital ID numbers, Patient Initials, Date of Birth etc.) in order to protect the anonymity of patients who have not provided written informed consent for this trial.

Inclusion Criteria

Patients were recruited from 19 Canadian cardiac surgical programs. We enrolled high-risk cardiac surgical patients who required one of the following high-risk surgical interventions, either on an elective or urgent basis (***NB surgery had to be performed on CPB***):

- re-operation for coronary artery bypass graft (CABG);

- re-operation for aortic valve replacement;
- re-operation for mitral valve replacement or repair;
- initial mitral valve replacement
- aortic and/or mitral valve replacement/repair with a CABG;
- multiple valve replacement/repair (initial or re-operation);
- ascending aortic artery procedures (including Bentall procedures, etc.).

Exclusion Criteria

Patients were excluded if they:

- were less than 19 years of age;
- refused consent (refusal from patient or physician);
- had a terminal illness with a life expectancy of less than 3 months;
- were previously enrolled in this study;
- were currently enrolled in another perioperative interventional study;
- were unable to receive blood products (i.e. difficulty with cross matching, inability to receive blood product, including Jehovah's Witnesses, or a past history of unexplained severe transfusion reaction);
- exposed to Aprotinin within 6 months prior to randomization
- have had a thrombocytopenia defined as a platelet count $<100,000/\text{mm}^3$;
- have had a coagulopathy defined as an INR > 1.5 prior to surgery or the Immediate preoperative use of tPA or streptokinase;
- have had a known or suspected adverse reaction to any of the study medications;
- were scheduled for a primary or re-operation for adult congenital heart procedures.
- had the possibility of mitral valve repair only

Pre-operative treatment with aspirin was not a contraindication to enrollment in the study.

Appendix B. Blinding maneuvers and co-interventions

Blinding maneuvers

All hospital pharmacies followed the same triple-dummy blinding scheme. They prepared a study package consisting of three 250-ml intravenous bags of normal saline, each containing the appropriate study drug as per the study dosing protocol. The first bag was given as an infusion over 10 minutes, the second was administered into the cardiopulmonary bypass circuit, and the third bag was used for maintenance infusion at a rate of 62.5 ml/h commencing once bypass was initiated and ending upon closure of the midline sternotomy. For operations exceeding 4 hours, a second infusion bag of 125 ml was dispensed.

To minimize selection and ascertainment biases, the anesthesiologists, surgeons, investigators, research staff, and members of the Data and Safety Monitoring Committee (an independent committee that monitored safety throughout the study) were all blinded to the randomization schemes and treatments administered. The trial statistician designated another statistician to prepare all randomization schemes and interim analyses. Only the designated research pharmacist in each hospital was aware of the treatment allocation for individual patients.

Unmasking of treatment allocation was allowed only under specified, exceptional circumstances when death or life-threatening complications would ensue if the study intervention was not revealed. In the event of excessive postoperative bleeding (200 ml/h for 3-4 hours) with or without repeat surgery requiring cardiopulmonary bypass, the attending physician or his or her designate could administer open-label antifibrinolytic agents at his or her discretion.

Co-interventions

Other than random allocation to either aprotinin, tranexamic acid or aminocaproic acid, there were no other interventions mandated as part of clinical care protocols. However, we recommended guidelines for the management of anticoagulation and transfusions. Heparin was infused to maintain a kaolin-based activated clotting time greater than 480 seconds during cardiopulmonary bypass. Protamine was administered in accordance to usual practice to return activated clotting time to normal after cardiopulmonary bypass.

Transfusion of red blood cells, one unit at a time, was suggested when hemoglobin concentrations fell below 65 g/L during cardiopulmonary bypass and 80 g/L after cardiopulmonary bypass. In the presence of blood loss exceeding 200 ml/h, transfusions could be administered as clinically indicated to replace blood volume. Fresh-frozen plasma was suggested if bleeding exceeded 200 ml/h, the international normalized ratio exceeded 1.5 or blood loss was considered excessive by clinical staff. Units of platelets from random donors were transfused if excessive blood loss resulted in a platelet count of less than 50,000/mm³.

Appendix C. Description of the deliberations of the Blood conservation in Anti-fibrinolytics in a Randomized Trial (BART) Data Safety Monitoring Board

The purpose of this document, written by the BART Data Safety Monitoring Board, is to describe the rationale for the recommendation by the DSMB that randomization of patients to receive aprotinin should be stopped on October 16, 2007.

The DSMB membership consisted of Dr. Andreas Laupacis (Chair), Dr. Jean-Francois Hardy, Dr. Conrad Pelletier and Professor Robin Roberts. Together, the DSMB had expertise in clinical trial methodology, statistics, cardiac anaesthesiology, cardiac surgery and general internal medicine.

Prior to the start of the trial, the DSMB agreed that it would receive summary information about efficacy and safety in the three treatment groups every three months, but that a formal interim analysis evaluating efficacy would only be performed twice: when one-third and two-thirds of patients had at least 30 days of follow-up in the trial. In the BART protocol it was indicated that the p values that the committee would use when considering stopping the trial for efficacy were 0.0003 and 0.0046 respectively. The primary outcome for efficacy was major bleeding, defined as greater than 1.5 litres of post-operative blood loss in 8 hours, transfusion of more than 10 units of red blood cells, re-operation for hemorrhage, or death due to hemorrhage. The sample size was a total of 2970 patients, equally randomized to aprotinin, tranexamic acid and epsilon-aminocaproic acid.

At the second interim analysis, in January 2007, when 30 day follow-up information was available for 1,896 patients, treatment A was associated with a lower risk of major bleeding compared with treatment B (relative risk (RR) 0.62, $p=0.005$; absolute risk 7.9% vs. 12.7%) and treatment C (RR 0.65, $p=0.01$; absolute risk 7.9% vs. 12.1%). Although statistically significant according to conventional hypothesis testing, the p -values were not below the boundary of 0.0046 that was previously established for early termination of enrolment of patients into BART (although the comparison of A versus B was extremely close).

At the same time, it was noted that the risk of death was greater in Drug A compared with the other two drugs: RR 1.39, $p=0.19$; absolute risk 5.0% vs. 3.9% for Drug A versus Drug B, and RR 1.48, $p=0.11$; absolute risk 5.0% vs. 4.3% for Drug A versus C. This trend in increased mortality was not statistically significant, but it raised a potentially serious concern about the safety of treatment A. The DSMB unblinded itself to Treatment A, and determined that it was aprotinin. The DSMB remained unaware of the identity of treatments B and C throughout the trial, except that they were not aprotinin.

When interpreting the mortality data, the DSMB considered the following information:

- Two recently published observational analyses of one cohort of 4374 post-operative cardiac patients^{1,2} had found an association between aprotinin treatment and an increased risk of peri-operative renal failure requiring dialysis (odds ratio (OR) 2.4), a 55% increase in myocardial infarction or heart failure, and a 181% increase in stroke or encephalopathy in “primary” surgery compared with tranexamic acid and aminocaproic acid. As well, the investigators found an increase in 5-year mortality associated with

aprotinin. The DSMB was concerned about the ability of the authors of these two observational studies to adequately adjust for the considerable differences in patient characteristics in the treatment groups in their study, and considered the magnitude of the increases in adverse events in aprotinin patients to verge on biological implausibility. Thus, the DSMB did not consider the results of these two studies convincing. However, the mortality results of BART were concerning when considered in conjunction with these two observational studies.

- The DSMB noted that there was no increase in renal failure requiring dialysis in aprotinin-treated patients in BART (1.7% in treatment A, 2.6% in treatment B, 1.6% in treatment C), nor in the frequency of stroke (3.1% in treatment A, 4.1% in treatment B, 2.7% in treatment C). The frequency of myocardial infarction (confirmed or suspected from the Serious Adverse Event form) was 4.4% in Treatment A, 3.5% in Treatment B and 2.8% in Treatment C. The proportions of patients with a post-operative serum tropinin level greater than five times the upper limit of normal were 48% in Treatment A, 52% in Treatment B and 53% in Treatment C.
- The DSMB examined the causes of death in the three BART treatment groups, and the causes of death in aprotinin-treated patients appeared similar to the causes of death in treatments B and C (in other words, there was no cluster of thrombotic deaths in aprotinin-treated patients).
- The DSMB felt that systematic reviews of previous randomized trials of aprotinin would provide information about mortality that was less biased than the observational studies mentioned above. The DSMB identified four such systematic reviews(3-6), and there was

no trend towards an increased frequency of death in aprotinin patients (compared with placebo, aminocaproic acid or tranexamic acid) in any of these studies.

Given this information, the DSMB felt that randomization of patients into BART should continue as planned because a) there was compelling evidence that aprotinin decreased the frequency of post-operative bleeding (a clinically important outcome), and b) the trend towards an increased risk in mortality might be due to chance (given multiple looks at the data, the fact that no clusters of aprotinin-associated thrombotic deaths were identified, and that previous systematic reviews of thousands of patients randomized to aprotinin did not show any trend towards an increase in mortality in aprotinin-treated patients when compared with placebo or an active comparator). However, the DSMB decided to statistically compare mortality among the three treatment groups every four months until the end of the trial.

In October 2007, the mortality analysis of 2,163 patients showed persistence of the increased risk of 30 day mortality in aprotinin patients, and the p values had approached conventional statistical significance (RR 1.5, $p=0.06$; absolute risk 6.5% vs. 4.2% comparing aprotinin with treatment B, and RR 1.5, $p=0.08$; 6.5% vs. 4.3% comparing aprotinin with treatment C). The benefit of aprotinin upon the primary outcome was similar to the results of the second interim analysis.

At this point, the DSMB recommended that randomization to the aprotinin arm of BART should be stopped.

The DSMB recognized that repeated looks at the data meant that the statistical strength of the association between aprotinin and mortality might be over-estimated. However, a comparison of aprotinin with a combination of treatments B and C would have yielded a p value less than 0.05 (B and C had similar risks of mortality). The DSMB also recognized that the evidence that aprotinin decreases serious post-operative hemorrhage was stronger than the evidence that it increases mortality. However, because all cause mortality is a much more serious outcome than serious post-operative bleeding, the DSMB believed that it was unlikely that clinicians would consider aprotinin to be an appropriate treatment for the vast majority of patients, given the results of the BART trial. Finally, continued enrolment of patients to achieve the planned sample size of 2970 would have been unlikely to significantly change the findings of the study.

Some clinicians and patients may feel that the best estimate of the effects of aprotinin upon mortality will be a systematic review combining BART with all previous RCTs. Given that previous systematic reviews have shown no trend towards an increase in mortality associated with aprotinin, it is likely that the systematic review including BART will find a non-statistically significant trend towards an increase in mortality. Some will find these results ambiguous. However, the most important responsibility of the DSMB was towards the patients entering into BART, and the DSMB felt that the BART mortality data were sufficiently concerning (both in terms of statistical significance and absolute risk) that equipoise no longer existed regarding the aprotinin arm of BART for the patients being approached about entry into the BART study.

Acknowledgement

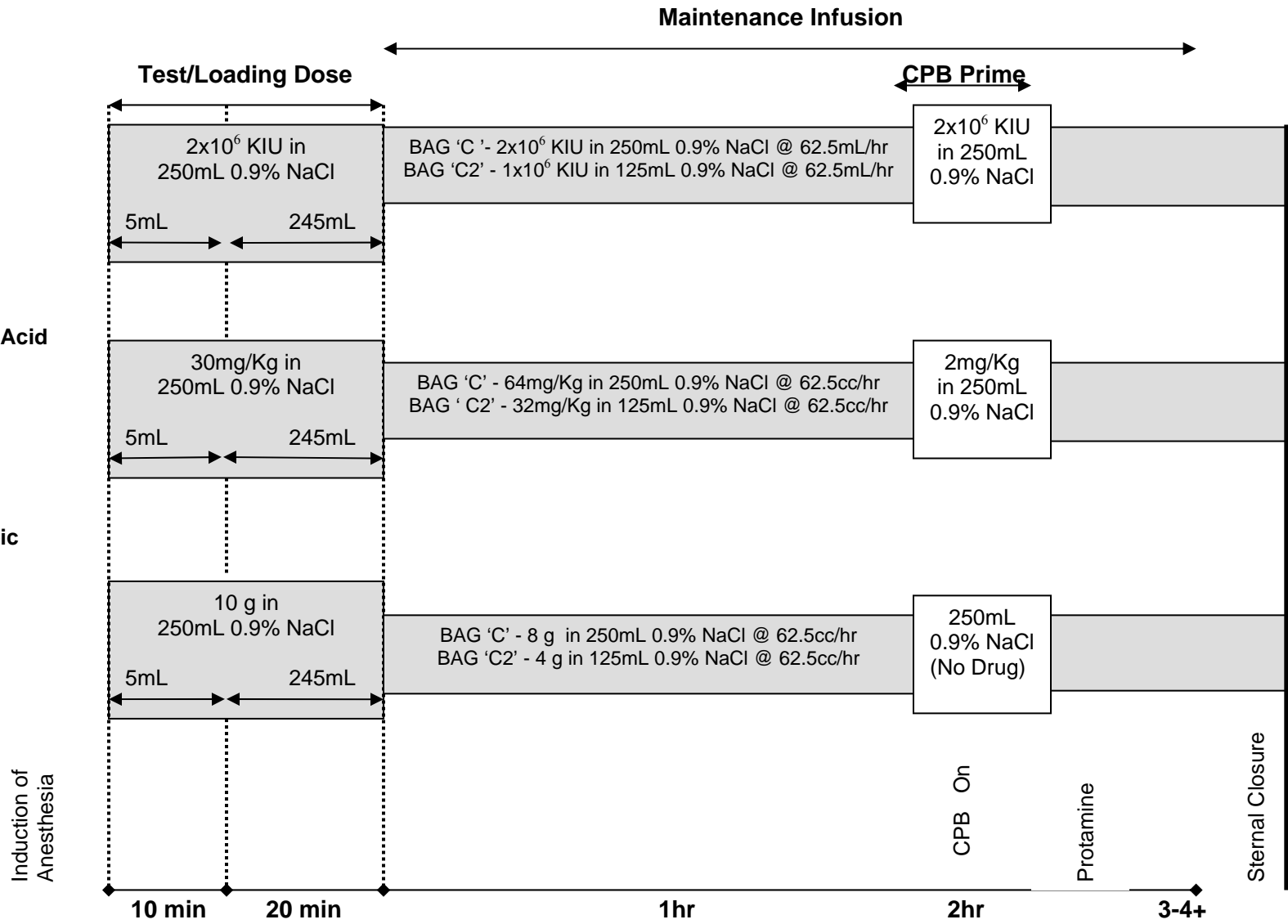
The DSMB would like to thank Jennifer Clinch for conducting the analyses that supported our work.

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Online Figure 1. Description of the drug administration and dosing scheme



Online Table 1: Massive bleeding in among the major subgroups among the 2330* cardiac surgical patients in the BART trial

Major Subgroup	Aprotinin		Tranexamic acid		Aminocaproic acid		Aprotinin vs Tranexamic acid		Aprotinin vs Aminocaproic acid	
	Events (%)	n	Events (%)	n	Events (%)	n	Relative Risk	95% CI	Relative Risk	95% CI
Total	74(9.5)	780*	93(12.1)	770	94(12.1)	780	0.79	(0.59,1.05)	0.80	(0.60,1.07)
Gender										
Male	56(10.3)	542	68(12.1)	562	68(12.0)	569	0.85	(0.61,1.19)	0.86	(0.62,1.21)
Female	18(7.6)	238	25(12.0)	208	26(12.3)	211	0.63	(0.35,1.12)	0.61	(0.35,1.09)
Age										
<65	26(8.8)	296	31(10.7)	290	35(11.5)	305	0.82	(0.50,1.35)	0.77	(0.47,1.24)
65 to <75	29(10.4)	280	34(12.8)	266	34(12.1)	282	0.81	(0.51,1.29)	0.86	(0.54,1.37)
75 to <80	15(10.2)	147	20(13.3)	150	17(12.6)	135	0.77	(0.41,1.44)	0.81	(0.42,1.56)
80+	4(7.0)	57	8(12.5)	64	8(13.8)	58	0.56	(0.18,1.77)	0.51	(0.16,1.60)
Type of Procedure										
Redo CABG	5(6.7)	76	6(6.4)	94	8(9.0)	89	1.03	(0.33,3.25)	0.73	(0.25,2.14)
CABG + ≥ 1 procedure	43(9.8)	438	54(12.7)	427	49(11.8)	417	0.78	(0.53,1.13)	0.84	(0.57,1.23)
Other	26(9.8)	266	33(13.25)	249	37(13.5)	274	0.74	(0.45,1.20)	0.72	(0.45,1.16)
Pre-op Aspirin										
None	34(8.6)	396	37(9.1)	409	37(9.3)	399	0.95	(0.61,1.48)	0.93	(0.59,1.44)
Any	40(10.6)	376	56(15.7)	357	57(15.1)	378	0.68	(0.46,0.99)	0.71	(0.48,1.03)
Co-morbid illnesses										
None	29(10.3)	283	22(8.2)	268	34(11.6)	293	1.25	(0.74,2.12)	0.88	(0.55,1.41)
Any	45(9.1)	497	71(14.1)	502	60(12.3)	487	0.64	(0.45,0.91)	0.73	(0.51,1.06)
Pre-op Hemoglobin (g/dl)										
< 11.0	8(14.0)	57	7(14.3)	49	6(12.5)	48	0.98	(0.38,2.51)	1.12	(0.42,3.01)
11.0 to 14.0	35(9.1)	385	54(13.4)	404	46(12.1)	380	0.68	(0.46,1.02)	0.75	(0.50,1.14)
> 14.0	31(9.2)	337	30(9.7)	310	41(11.8)	348	0.95	(0.59,1.53)	0.78	(0.50,1.21)
ASA pre-op score (1)										
< 4	30(9.5)	317	44(12.8)	345	43(12.5)	344	0.74	(0.48,1.15)	0.76	(0.49,1.18)
≥ 4	43(10.2)	421	44(11.7)	377	45(11.7)	384	0.87	(0.59,1.30)	0.87	(0.59,1.29)

(1) ASA is the American Society of Anesthesiologists

* No information post surgery was available for one patient in the Aprotinin group. However, survival status and 30 day information was available.

Online Table 2. Primary outcome of massive bleeding compared using 97.5 percent confidence intervals

Component of the primary composite outcome	Aprotinin N=780*	Tranexamic acid N=770	Aminocaproic acid N=780	Aprotinin vs Tranexamic acid		Aprotinin vs Aminocaproic acid	
	Events (%)	Events (%)	Events (%)	Relative Risk	97.5% CI	Relative Risk	97.5% CI
Components of the Outcome							
Bleeding from chest tubes	41 (5.3)	58 (7.5)	65 (8.3)	0.70	(0.45,1.09)	0.63	(0.41,0.97)
Massive transfusion	16 (2.1)	17 (2.2)	22 (2.8)	0.93	(0.43,2.01)	0.73	(0.35,1.51)
Death due to hemorrhage	11 (1.4)	8 (1.0)	4 (0.5)	1.36	(0.48,3.82)	2.75	(0.75,10.13)
Re-operation for bleeding	43 (5.5)	62 (8.1)	64 (8.2)	0.68	(0.45,1.05)	0.67	(0.44,1.03)
Massive bleeding	74 (9.5)	93 (12.1)	94 (12.1)	0.79	(0.56,1.09)	0.79	(0.57,1.09)

* No information post surgery was available for one patient in the Aprotinin group. However, survival status and 30 day information was available.

Online Table 3: Thirty-day all cause mortality rates overall and in major subgroups among the 2328* patients in the BART trial

Characteristic	Aprotinin		Tranexamic acid		Aminocaproic acid		Aprotinin vs Tranexamic acid		Aprotinin vs Aminocaproic acid	
	Events (%)	n	Events (%)	n	Events (%)	n	Relative Risk	95% CI	Relative Risk	95% CI
Total	47(6.0)	779	30(3.9)	769	31(4.0)	780	1.55	(0.99,2.42)	1.52	(0.98,2.36)
Gender										
Male	28(5.2)	541	17(3.0)	562	22(3.9)	569	1.71	(0.95,3.10)	1.34	(0.78,2.31)
Female	19(8.0)	238	13(6.3)	207	9(4.3)	211	1.27	(0.64,2.51)	1.87	(0.87,4.05)
Age										
<65	14(4.7)	296	4(1.4)	289	8(2.6)	305	3.42	(1.14,10.26)	1.80	(0.77,4.24)
65 to <75	18(6.5)	279	10(3.8)	266	8(2.8)	282	1.72	(0.81,3.65)	2.27	(1.00,5.14)
75 to <80	9(6.1)	147	6(4.0)	150	10(7.4)	135	1.53	(0.56,4.19)	0.83	(0.35,1.97)
80+	6(10.5)	57	10(15.6)	64	5(8.6)	58	0.67	(0.26,1.74)	1.22	(0.39,3.78)
Type of Procedure										
Redo CABG	8(10.7)	75	4(4.3)	94	4(4.5)	89	2.51	(0.78,8.01)	2.37	(0.74,7.57)
CABG + ≥ 1 procedure	27(6.2)	437	17(4.0)	427	20(4.8)	417	1.55	(0.86,2.81)	1.29	(0.73,2.26)
Other	12(4.5)	267	9(3.6)	248	7(2.6)	274	1.24	(0.53,2.90)	1.76	(0.70,4.40)
Pre-op Aspirin										
None	25(6.3)	395	15(3.7)	408	8(2.0)	399	1.72	(0.92,3.22)	3.16	(1.44,6.91)
Any	22(5.9)	376	15(4.2)	357	23(6.1)	378	1.39	(0.73,2.64)	0.96	(0.55,1.69)
Co-morbid illnesses										
None	14(5.0)	283	3 (1.1)	267	6(2.1)	293	4.40	(1.28,15.15)	2.42	(0.94,6.20)
Any	33(6.7)	496	27(5.4)	502	25(5.1)	487	1.24	(0.76,2.03)	1.30	(0.78,2.15)
Pre-op Hemoglobin (g/dl)										
< 11.0	10(17.5)	57	4(8.2)	49	4(8.3)	48	2.15	(0.72,6.42)	2.11	(0.70,6.29)
11.0-14.0	22(5.7)	384	21(5.2)	403	22(5.8)	380	1.10	(0.61,1.97)	0.99	(0.56,1.76)
> 14.0	15(4.5)	337	5(1.6)	310	5(1.4)	348	2.76	(1.01,7.50)	3.10	(1.14,8.43)
ASA pre-op score (1)										
< 4	16(5.1)	316	8(2.3)	345	11(3.2)	344	2.18	(0.95,5.04)	1.58	(0.75,3.36)
≥ 4	30(7.1)	421	20(5.3)	377	17(4.4)	384	1.34	(0.78,2.32)	1.61	(0.90,2.87)

(1) ASA is the American Society of Anesthesiologists

* No follow up information was obtained for 3 patients due to patients that withdrew consent. (2 in the Aprotinin group and 1 in the Tranexamic acid group)

Online Table 4: Blood Products Administered following cardiac surgery among the 2330* cardiac surgical patients in the BART trial

Blood product	Aprotinin		Tranexamic acid		Aminocaproic acid		Aprotinin vs Tranexamic acid		Aprotinin vs Aminocaproic acid	
	Events (%)	n	Events (%)	n	Events (%)	n	Relative Risk	95% CI	Relative Risk	95% CI
Platelets	217(27.8)	780	248(32.3)	770	291 (37.3)	780	0.86	(0.74,1.00)	0.75	(0.65,0.86)
Fresh frozen plasma	275(35.3)	780	291(37.8)	770	354 (45.4)	780	0.93	(0.81,1.06)	0.78	(0.69,0.88)
Cryoprecipitate	48(6.2)	780	66(8.6)	770	72 (9.3)	780	0.72	(0.50,1.03)	0.67	(0.47,0.95)
Red blood cells	419(53.7)	780	506(65.7)	770	514 (66.9)	780	0.82	(0.75,0.89)	0.81	(0.75,0.88)

* No information post surgery was available for one patient in the Aprotinin group. However, survival status and 30 day information was available.