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Association of the 98T ELAM-1 Polymorphism with Increased Bleeding After Cardiac Surgery

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INTRODUCTION

Postoperative coagulopathy, bleeding and high blood product usage remains a serious problem in cardiac surgical patients.^{1,2} It is also relatively common, as up to 10% of cardiac surgical patients will experience major blood loss that is associated with increased morbidity and mortality;³ about one third of these patients require reoperation for hemorrhage.² While contemporary mortality rates for coronary artery bypass graft (CABG) surgery are approximately 2%, mortality in patients with coagulopathic bleeding increases to over 10%.^{3,4} Unfortunately, current preoperative testing strategies to identify patients at high risk of postoperative hemorrhage are poorly predictive.²

Alterations in the hemostatic system during cardiac surgery are multifactorial, including the effects of hypothermia, hemodilution, and cardiopulmonary bypass (CPB) circuit-induced activation of coagulation, fibrinolytic and inflammatory pathways. Tipping the balance from normal hemostasis to coagulopathic bleeding may be determined, in part, by an individual variation in any of the above pathways and genetic variability in coagulation proteins and platelet glycoproteins has been shown to affect bleeding after cardiac surgery.^{5–7}

Activation of the coagulation system during CPB is primarily mediated by tissue factor (TF)⁸ and expression of TF, primarily on monocytes and endothelium, is modulated by cytokines (interleukins-1, 6 and 8/IL-1, IL-6, IL-8, tumor necrosis factor/TNF)^{9,10} and adhesion molecule dependent platelet/leucocyte/endothelium interactions (P-selectin/GMP140/CD62P, endothelial leucocyte adhesion molecule -1/ELAM-1 (or E-selectin), intracellular adhesion molecule/ICAM-1).^{11,12} Furthermore, the magnitude of cytokine release is genotype dependent^{13–16}, as is the modulation and disease association of adhesion molecules.^{17,18}

Therefore genetic variation in the inflammatory pathway may determine the response of the inflammatory system to CPB and affect the development of CPB related coagulopathy. This present study aims to investigate the association of multi-locus genetic variability with postoperative bleeding using a candidate gene approach, by testing the hypothesis that established polymorphisms of IL-1, IL-6, IL-8, TNF α , P-selectin, ELAM-1 and ICAM-1 genes are associated with bleeding following CABG surgery.

METHODS

Study Population

This analysis represents a sub-study of the Perioperative Genetics and Safety Outcomes Study (PEGASUS), an ongoing IRB approved, prospective, longitudinal study at Duke University Medical Center, having enrolled 3149 patients who have given written informed consent to have their clinical and genetic data analyzed in relation to perioperative outcomes.

Using a pilot study of 100 patients we estimated sample size based on a two group 2-sided t-test of equal means, stipulating 80% power and an alpha level of 0.05. We estimated that 698 patients would be required to detect a 30% increase in postoperative chest tube drainage (CTD) due to the presence of an allele which occurs in the population at a rate of 10% (minor allele frequency = 0.10), using nQuery Advisor Release 4.0. We therefore limited candidate polymorphisms to those with a minor allele frequency > 9%. Our study population included 877 patients of the 3149 genotyped PEGASUS cohort who underwent primary non-emergent CABG surgery using CPB between January 2000 and April 2002, as previously described,⁵ and should be considered a reanalysis of this dataset with some overlap of patients from both analyses being included in the final model.

Inclusion criteria were adult, primary, non-emergent CABG surgery and exclusion criteria were liver disease, end-stage renal disease, preexisting coagulopathy (including anti-platelet agents other than aspirin or thrombolytic therapy) and cardiogenic shock. Patients undergoing reoperation for bleeding were also excluded, as the cause (surgical versus non-surgical) was not detailed in the database.

Patient data collection and definition of phenotypes

Blood was collected prior to anesthetic induction for isolation of genomic DNA. Prior to initiation of CPB all patients received heparin anticoagulation (300–400 units/kg) to achieve a target activated clotting time (Actalyke, Helena Laboratories, Beaumont, TX) over 480 seconds and intravenous ϵ -aminocaproic acid (10g bolus and 1g/hour for 5 hours). Non-pulsatile, hypothermic (28–34°C) CPB was conducted using a membrane oxygenator (Cobe CML, Cobe Inc., Arvada, CO) following a crystalloid and mannitol prime and using an arterial line filter (Cobe). Porcine heparin was supplemented during CPB, as needed, to maintain an activated clotting time over 480 seconds. During CPB, temperature adjusted flow rates of 2.5l/min/m² were used and mean arterial pressure was generally maintained between 50–60 mmHg, or 60–70 mmHg if previous evidence of cerebrovascular disease was noted. Anesthesia was maintained using isoflurane (0.5–1.0%) via the oxygenator. Alpha stat management for maintenance of normal pH, pO₂ and pCO₂ values was used. A hematocrit of 0.18 to 0.20 was typically acceptable during CPB but this would be increased to >0.20 for separation from CPB. Cold blood or crystalloid cardioplegia solution was used for myocardial protection and infused either antegrade, retrograde or antegrade/retrograde according to the surgeon's preference and the clinical circumstance. The BRAT cell salvage device (Cobe) was used in preference to cardiotomy suction, unless brisk bleeding from a cardiac chamber was encountered, and was used pre and post CPB until the skin was closed.

After separation from CPB, protamine sulphate, up to 1mg for every 100 units of heparin given, was used to reverse heparin anticoagulation.

The primary outcome variable was postoperative chest tube drainage, reflecting bleeding from the mediastinal and thoracic cavities, during the first 4 hours after intensive care unit admission (CTD). This time point was chosen due to its established relationship with laboratory markers of coagulopathy in our population.¹⁹ Perioperative transfusion requirements and laboratory measures of coagulation (platelet count, prothrombin time and activated partial thromboplastin time) were also described. The transfusion of blood products was guided by laboratory testing consistent with the recommendations of the American Society of Anesthesiologists.²⁰

Rationale for Candidate Polymorphism Selection

Primers were designed for 37 polymorphisms on 11 genes, previously associated with a proinflammatory phenotype and with a high likelihood of functional effect (nonsense or missense, non-synonymous variants or frame shift insertions/deletions in the coding sequence, or mutations in the promoter or control sequence of a gene). Eighteen polymorphisms in 8 candidate genes (TNF, IL-1, IL-6, IL-8, IL-10, interleukin 1 receptor antagonist/IL-1Ra, ICAM-1 P-selectin and ELAM-1) were selected on the basis of a minor allele frequency of > 9% in the pilot sample, and are detailed in Table 2.

Isolation of Genomic DNA and Genotype Analysis

Genomic DNA extraction from whole blood collected preoperatively was performed using the Puregene™ system (Gentra Systems, Minneapolis, MN), quantitated, and stored at 4°C under chloroform. Genotyping assays for single nucleotide polymorphisms (SNPs) were conducted at Agencourt Bioscience Corporation (Beverly, MA) by Matrix Assisted Laser Desorption/Ionization, Time-Of-Flight (MALDI-TOF) mass spectrometry, using the Sequenom™ MassARRAY™ system (Sequenom, San Diego, CA).²¹ Genotyping accuracy of the Sequenom™ MassARRAY™ system was estimated at 99.6%.²² Reproducibility of genotyping was validated to be >99% by scoring a panel of 6 polymorphisms in 100 randomly selected patients using direct sequencing on an ABI3700 capillary sequencer (Applied Biosystems, Foster City, CA). Genetic samples were linked to covariate and phenotypic variables in a relational database with extensive quality control features, and deidentified to ensure patient confidentiality.

Statistical Analysis

Descriptive statistics were calculated using SAS/Genetics system version 8.02 (SAS Inc, Cary, NC). Unadjusted CTD data were determined by genotype (ELAM-1 gene 98GG, 98GT or 98TT) and presented as median [interquartile range] (median [IQR]). Excessive CTD was defined as values falling in the upper quartile of the whole sample. Blood product transfusion was described in a similar way but 98GG was described as wild type and 98GT and 98TT collapsed into a minor allele group.

Allele frequencies were calculated by the allele-counting method; Hardy-Weinberg equilibrium at each locus and linkage disequilibrium between markers within the same gene were assessed by the χ^2 test.²³ Prior to multivariate analysis, homozygous and heterozygous genotypes for the minor allele were combined. Consequently, analyses are based on 2 genotypic classes for each candidate polymorphism, reflecting the presence (1 or 2 copies) or absence of the minor allele. The primary analysis is the multivariate association of genotype with 4-hour CTD

Since the primary outcome data were not normally distributed, logarithmic transformation was applied to 4-hour CTD for inclusion in multivariate linear regression models. A *clinical* linear regression model relating perioperative and demographic variables previously shown to account for variation in postoperative bleeding was first developed. While our final model contained age, weight, height, diabetes, preoperative hemoglobin and platelet count, the following variables were tested for association with 4-hour CTD with univariate models : body mass index, weight, height, age, total heparin dose, total protamine dose, heparine/protamine ratio, preoperative hemoglobin and platelet count, diabetes, duration of cardiopulmonary bypass, duration of aortic cross-clamp, activated clotting times (maximum and last), volumes of cell saver, crystalloid and colloid administered. Only significant predictors ($p < 0.05$) were included as covariates in the final multivariable models.

Then, for each polymorphism, univariate analyses were performed testing the null hypothesis of no association between genotype and postoperative CTD. An association with a p -value < 0.10 included a polymorphism in a multiple linear regression model for log of 4-hour chest tube drainage and the resulting combination of individual polymorphisms constituted our *genetic* model. Finally, a *combined* model, adding independent variables from the *genetic* model to those already identified in the *clinical* model, was also fit to determine the extent to which genetic polymorphisms account for variation in CTD beyond that explained by clinical variables. The Bonferroni correction was used to account for multiple testing and set the alpha level at $p < 0.0029$ for genetic data. Individual polymorphisms with this level of association with bleeding were considered potentially clinically relevant and examined for the secondary outcomes of laboratory evidence of coagulopathy and transfusion.

Estimated regression coefficients were used to test the associations of haplotypes of relevant SNPs with bleeding, as previously described.²⁴ We have previously demonstrated that potential bias from population admixture²⁵ does not affect bleeding in our cardiac surgery population⁵ so this was not included in the current analysis.

RESULTS

Characteristics of the study population (demographics and clinical covariates) are listed in Table 1. Of the original 877 patients, there were 97 exclusions: Patients with liver disease ($n=8$), end-stage renal disease ($n=48$), preexisting coagulopathy ($n=6$), reoperation for bleeding ($n=26$), and cardiogenic shock ($n=9$) were excluded; patients receiving clopidogrel had the drug discontinued for 5–7 days prior to surgery and no patients received thrombolytic or GPIIb/IIIa antagonists within 72 hours preoperatively. Of the remaining 780 patients, genotype data was available in 759 patients.

The genotypes of the 18 candidate polymorphisms are detailed in Table 2. Of note, the minor alleles of all the polymorphisms occur commonly in our sample of cardiac surgery patients, where minor refers to the less common allele of a polymorphism in the general population; the common allele is described as the wild type. The minor alleles of 7 of the 18 candidate polymorphisms occurred in over 50% of patients, ranging from 17 to 87% of patients.

There was a strong correlation between CTD for the first 4 and 12 postoperative hours ($r = 0.85$, $p < 0.0001$) and for the multivariate regression model we used 4 hour CTD as stated *a priori* in the methods section. Both 4 and 12 hour CTD are included in Figure 1. Three polymorphisms (ELAM-1 98G/T, ICAM-1 778G/A and IL-10 -819C/T) were associated with CTD on univariate testing and were included in the multivariate genetic model. We identified clinical factors relating to patient size and preoperative hemoglobin concentration

that were associated with CTD (Table 3), and, after adjusting for the clinical factors, only the ELAM-1 98G/T and IL-10 -819C/T SNPs were independently associated with CTD. Following Bonferroni correction for multiple testing, only ELAM-1 98G/T was independently associated with CTD (F-value 9.73; $p=0.0019$).

Unadjusted values of CTD are presented in Figure 1 by genotype of the ELAM-1 gene 98GT SNP (wild type GG, GT and TT). As shown in Figure 1, 4hr CTD and 12hr CTD was 215 [142–320] milliliters and 525 [355–755] milliliters for wild type (GG), 250 [180–390] milliliters and 570 [425–880] milliliters for heterozygotes (GT) and 257 [210–540] milliliters and 702 [515–975] milliliters for the rare, homozygous variants (TT). Excessive CTD (defined as falling in the upper quartile of the whole sample) was observed for 24%, 31% and 40% of patients with the GG, GT and TT genotypes respectively. After adjusting for the clinical factors in a multivariate analysis, a gene dose effect for the 98T allele was observed, with heterozygotes (GT) bleeding 17% more and homozygotes (TT) bleeding 54% more than wild type (GG) genotypes respectively ($p=0.01$, in a regression analysis testing for the effect of number of minor alleles present).

With regards to laboratory test results immediately after surgery (see Table 4), there were no differences in hemoglobin concentration, platelet count, prothrombin time, fibrinogen level or white blood cell count by genotype. However, a higher postoperative activated partial thromboplastin time (APTT) was noted in carriers of the 98T allele of the ELAM-1 gene (genotypes 98GT and 98TT; $p=0.0023$). However, this did not lead to a marked change in transfusion practice as red cell transfusion was 2[0–4] units for both wild type and minor allele carriers and there was no difference in amounts of individual products transfused to wild type compared to 98T carriers (Table 5). Excessive transfusion (> 5 units of PRBCs) was seen in 26% of wild types and 32% of minor allele (98T) carriers and when platelet adult doses/apheresis units, FFP units or cryoprecipitate 10 dose packs were combined as “hemostatic blood products” 40% of 98T carriers received hemostatic blood products compared to 31% for wild type patients.

Haplotype analysis was performed for the two ELAM polymorphisms included in the candidate SNPs (98G/T and 561A/C). Estimates of haplotype frequencies are given in Table 6. The G-A and T-C haplotypes account for approximately 98% of haplotypes for these two SNPs. Estimated regression coefficients, under a default additive model of haplotype effects, are given for haplotypes C-G, T-A, and T-C, with common haplotype G-A serving as the reference haplotype. Haplotype T-A ($p = 0.01$) and haplotype T-C ($p = 0.03$) are associated with more bleeding than haplotype G-A.

Discussion

Our findings demonstrate that polymorphism of the ELAM-1 gene is associated with the development of postoperative coagulopathy and increased bleeding after CABG surgery. This genetic variation is independent of and additive to clinical factors when accounting for variability in postoperative bleeding.

Current knowledge of the effect of genetic variability on bleeding and transfusion after cardiac surgery is still limited but its relevance in preoperative risk stratification is being recognized. In a related analysis, we found an angiotensin converting enzyme polymorphism and co-possession of certain platelet surface glycoprotein and coagulation factor polymorphisms (GPIIaIIa, GPIb alpha, tissue factor, tissue factor pathway inhibitor and prothrombin) to be associated with bleeding after cardiac surgery.⁵ Donahue and colleagues⁶ reported that carriers of the Factor V Leiden polymorphism (a polymorphism associated with activated protein C resistance) had significantly reduced postoperative

bleeding and blood transfusion after cardiac surgery. However, the Factor V Leiden polymorphism is uncommon, with only 5% of a Tennessee sample⁶ and 2% of a North Carolina sample⁵ testing as heterozygotes for the Factor V Leiden mutation. In contrast, 17% of our population carried the minor, T allele of the ELAM-1 98G/T polymorphism, placing almost 1 in 5 of our patients at higher risk of bleeding.

ELAM-1 (or E-selectin) is one of the three members of the selectin family; the ELAM-1 gene contains 14 exons spanning about 13 kilobases of DNA on the q12 > qter region of human chromosome 1. Expression is induced by TNF, via NF-kappaB,²⁶ and the gene promoter region contains NF-kappa B- and AP-1-binding sites.²⁷ ELAM-1 is an adhesion molecule that is expressed on the surface of activated endothelial cells, supporting the rolling of leukocytes on activated endothelial cells^{28,29} and participating in the transition to stable adhesion that precedes transmigration.^{30,31}

ELAM-1 has been functionally linked to thrombosis. In knockout mice, ELAM-1 deficiency reduces the amount of fibrin incorporated into a thrombus,³² the mass of thrombus induced by vein ligation³² or the mass of thrombus induced by a model procoagulant state.³³ In humans, genetic variation in ELAM-1 modulates the extent of thrombin generation in response to the inflammatory stimulus of experimental endotoxemia³⁴ and alters the risk of venous thrombosis,³⁵ supporting a role of ELAM-1 in procoagulant mechanisms. Although, how the biology of ELAM-1 and the 98G/T polymorphism affects the APTT and postoperative bleeding is unknown.

The major limitation of this study is that ELAM-1 is a polymorphic gene that demonstrates linkage disequilibrium (LD),³⁶ such that expression of an allele from one polymorphism associated with a clinical finding, may be acting as a marker for an allele of another ELAM-1 polymorphism that confers the actual biological effect, thereby confounding statistical associations. Our haplotype analysis demonstrated LD between the 98G/T polymorphism, located in the gene promoter region but of unknown function, and the 561A/C polymorphism from the coding region of the gene but confirmed that the 98T allele had the stronger association with bleeding (Table 5). Despite this LD, a third, untested SNP could actually be the conveyor of the biological effect; for example 1402C/T³⁷ or Leu554Phe.³⁸ ELAM-1 SNPs have also been noted to be in LD with other selectin molecules on the same gene, such as L-selectin,³⁹ further complicating the potential for LD.

Further limitations include reanalysis of a previously reported dataset with overlap of patients included in both analyses, questionable broader applicability of a single, tertiary referral center finding and the a priori decision to exclude re sternotomy for bleeding as outliers with massive blood loss, when some of these patients may have excessive coagulopathic bleeding. As such, these findings should be regarded as preliminary and confirmed in subsequent studies.

By focusing on a predefined population, controlling for clinical risk factors for bleeding, testing multiple, common candidate polymorphisms chosen for biological plausibility, and choosing an endpoint with an established relationship to coagulopathic bleeding in this specific population,¹⁹ we attempted to optimally examine this complex phenotype. As is common with complex traits or phenotypes, the differences in outcome associated with individual polymorphisms are often relatively small, as shown in Figure 1. However, to put this finding into perspective, antifibrinolytic use was introduced as almost universal standard practice for reducing bleeding after cardiac surgery based on a 35% reduction of blood loss for ϵ -aminocaproic acid and a 53% reduction for high dose aprotinin.⁴⁰ We showed a 17% increase in bleeding in the GT heterozygotes and a 52% increase for TT homozygotes, and we detected this increased bleeding despite the routine use of ϵ -aminocaproic acid.

However, the magnitude of the absolute changes in bleeding that we observed in this population may be more clinically relevant, and lead to an increase in RBC and other blood product transfusion, if observations were extended to higher risk populations or more prolonged, complex surgeries that may result in an exaggerated coagulopathy.

In summary, our findings indicate that genetic variation in the ELAM-1 gene is associated with coagulopathy and bleeding after cardiac surgery. With the minor allele carried by almost 1 in 5 patients, preoperative genetic testing may have the potential to better stratify the risk of bleeding after cardiac surgery.

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ELAM-1 gene 98GT polymorphism variants and bleeding

Figure 1.

Bleeding, described as chest tube drainage at 4 and 12 hour postoperative timepoints, expressed graphically as median and interquartile range and tabulated as number and percentage of patients, by genotype, demonstrated excessive drainage defined as the upper quartile of the whole sample (n=668). Patients are categorized as wild type (GG) and carriers of one (GT) and two (TT) minor alleles of the ELAM-1 gene 98GT polymorphism.

Figure 2.

Table 1**Demographics**

Characteristics of the study population (n=780 individuals).

Demographic Variables	
Age (years)	63 (+/-11)
Gender (% female)	30
Race (% Caucasian)	80 *
Height (cm)	171 (+/-14)
Weight (Kg)	86 (+/-19)
Coexisting Conditions	
Diabetes (%)	35
Hypertension (%)	72
Current smokers (%)	31
Previous myocardial infarction (%)	26
Congestive cardiac failure (%)	16
Previous stroke (%)	6
Preoperative Renal Dysfunction (Cr>113mmol/L) (%)	5
Preoperative Bleeding Risk Factors	
Preoperative aspirin use (%)	83
Preoperative heparin infusion (%)	42
Preoperative platelet count ($\times 10^6/\text{ml}$)	213 (+/-68)
Preoperative hemoglobin (g/dl)	13.3 (+/-1.7)
Procedural Factors	
Number of bypass grafts	3.3 (+/-0.8)
Cardiopulmonary bypass duration (minutes)	108 (+/-42)
Total heparin dose (units)	39000 (+/-16000)
Total protamine dose (mg)	290 (+/-100)
Packed red blood cells transfused (units)	1.2 (+/-1.4)
Fresh frozen plasma transfused (ml)	75 (+/-260)
Platelets transfused (ml)	47 (+/-134)

* 15% of the study population are African-American. The remaining 5% were self-reported as Hispanic, Native American, or Asian. Continuous variables are expressed as mean $\pm$ standard deviation; categorical variables are presented as percent.

Abbreviations

mmol/l	micromoles per liter
g/dl	grams per deciliter
ml	milliliters
mg	milligrams

Candidate gene polymorphisms

Table 2

Major (wild-type) alleles are indicated to the left and minor alleles to the right of the/character. The function of polymorphisms associated with bleeding is discussed in the text.

Gene (synonyms)	SNP	Wild type	Genotype frequency		Univariate Analysis	
			Heterozygote	Homozygote	P value	n
Adhesion molecules						
ELAM-1	98G/T	83	15	2	0.006	668
	561A/C	83	16	1	0.033	716
ICAM-1	1462 A/G	18	41	41	0.036	739
	778G/A	78	20	2	0.026	743
P-selectin	−1969A/G	18	41	41	NS	
	1902A/G	41	43	16	NS	
	2013G/T	67	29	4	NS	
	2361A/C	72	24	4	NS	
	1087G/A	81	18	1	NS	
Proinflammatory cytokines						
TNF α	−308G/A	70	27	3	NS	
IL-1 β	5810G/A	55	33	12	NS	
	5887C/T	61	34	5	NS	
IL-6	−511 T/C	13	46	41	NS	
	−174G/C	37	47	16	NS	
	−597G/A	39	46	15	NS	
Antinflammatory cytokines						
IL-10-	319C/T	58	33	9	0.015	646
IL1-RN (IL1-Ra)	11100 T/C	48	42	10	NS	
	8006T/C	59	35	6	NS	

Abbreviations

SNP single nucleotide polymorphism

Abbreviations	
NS	Not significant (p > 0.10)
ELAM -1	Endothelial-leucocyte adhesion molecule-1
ICAM -1	Intracellular adhesion molecule-1
TNF α	Tumour necrosis factor α
IL-1 β	Interleukin -1 β
IL-6	Interleukin -6
IL-10	Interleukin -10
IL-1-RN (IL-1 -Ra)	Interleukin-1 receptor antagonist

Table 3
Multivariate model combining clinical and genetic effects

Results of multivariate linear regression models testing the association of inflammatory and adhesion molecule polymorphisms and clinical covariates with 4 hour postoperative chest tube drainage. Variables identified through independent assessments of clinical and genetic models were entered into the combined clinical and genetic variables model, shown above.

Combined model	n=502	R2 of 0.095
Polymorphism	F value	P value
ELAM -1 98G/T	9.73	0.0019
IL-10 -819C/T	6.38	0.0119
<i>Clinical factors</i>		
Weight	7.62	0.006
Height	11.35	0.0008
Preoperative Hb	6.92	0.0088

Abbreviations:

Hb	Hemoglobin concentration (g/dl)
ELAM-1	Endothelial-leucocyte adhesion molecule-1
IL-10	Interleukin-10

Table 4**Laboratory results**

Laboratory test results are expressed as median (interquartile range) and compared with Wilcoxon rank sum tests.

	Wild type (GG)	GT or TT	p value
APTT (seconds)	35.5 (30.9–40.4)	38.4 (33.2–43.5)	0.0023
Hemoglobin (g/dl)	9.90 (9.1–10.9)	10.0 (8.9–10.9)	0.98
PT (seconds)	1.4 (1.3–1.6)	1.4 (1.3–1.7)	0.61
Fibrinogen (g/dl)	212 (164–269)	208 (162–267)	0.73
Platelet count ($\times 10^9$)	123 (97–159)	111 (91–155)	0.30
White cell count ($\times 10^9$)	11.6 (8.9–15.2)	12.1 (8.7–14.9)	0.62

Abbreviations

APTT Activated partial thromboplastin time (seconds)

g/dl Grams per deciliter

PT Prothrombin time (seconds)

Table 5
Transfusion by genotype

	Whole Sample (n=668)	ELAM-1 gene 98GG Wild type (n=555)	ELAM-1 gene 98GT+98TT 98T carriers (n=113)
RBC			
Any given (n,%)	372 (59%)	307 (59%)	65 (58%)
Units given (median, IQR)	2 (0–4)	2 (0–4)	2 (0–4)
Excessive RBC use (n,%)	178 (27%)	142 (26%)	36 (32%)
Total non-RBC products			
Any given (n,%)	219 (33%)	174 (31%)	45 (39%)
Units given (median, IQR)	2 (0–6)	2 (0–6)	3 (0–7)
Plasma			
Any given (n,%)	192 (29%)	149 (27%)	43 (38%)
Units given (median, IQR)	0 (0–1)	0 (0–2)	0 (0–2)
Platelets			
Any given (n,%)	161 (24%)	129 (23%)	32 (28%)
Units given (median, IQR)	0 (0–0)	0 (0–1)	0 (0–1)

Abbreviations

RBC Red blood cell units

IQR Interquartile range (25th–75th percentile)

Non-RBC Plasma, platelets and cryoprecipitate units

Table 6**Haplotype analysis**

The frequency of occurrence of the 4 possible haplotypes of the two ELAM-1 polymorphisms (98G/T and 561 A/C) is tabulated as is the comparison of bleeding between the less common haplotypes and the common, reference haplotype G-A.

ELAM-1 98G/T	ELAM-1 561A/C	Haplotype frequency	p value
G	C	0.004	NS
T	A	0.012	0.01
T	C	0.083	0.03
G	A	0.901	NA

Abbreviations

ELAM-1 Endothelial-leucocyte adhesion molecule-1

NS Not significant

NA Not applicable