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Correlation of Pre-Operative Plasma Protein Concentrations in Cardiac Surgery Patients with Bleeding Outcomes Using a Targeted Quantitative Proteomics Approach

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Abstract

Purpose—Despite recent advancements in the use of thrombelastography (TEG) in the surgical setting, adequate technology to accurately predict bleeding phenotypes for patients undergoing cardiopulmonary bypass on the basis of non-mechanical parameters is lacking. While basic science and translational studies have provided key mechanistic insights about the protein components of coagulation cascades and regulatory mediators of hemostasis and fibrinolysis, targeted protein assays are still missing and the association of protein profiles to bleeding phenotypes and TEG readouts have yet to be discovered.

Objective—To identify protein biomarkers of bleeding phenotypes of cardiopulmonary bypass patients in pre-operative plasma.

Experimental design—We applied a targeted proteomics approach to quantify 123 plasma proteins from 23 patients undergoing cardiopulmonary bypass (CPB) and sternotomy. We then correlated these measurements to bleeding outcomes and TEG parameters, associated with speed of clot formation and strength.

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Conflicts of interest

The authors have declared no conflicts of interest relevant to this study.

Authors' contribution

NW collected the samples. NC, AT, ADA performed data analysis. AC, MF, MK and ADA performed statistical analysis. MD, KCH set up the method and performed proteomics analysis and Skyline-based raw data analyses. ADA prepared the figures. MD and KCH prepared supplementary Tables. ADA wrote the manuscript and all the authors critically contributed to its finalization. AB, NW, KCH supervised the experimental design, the realization of the experiments and the finalization of the study.

Results—In this pilot study, we demonstrate the feasibility of protein quantitation as a viable strategy to predict low versus high bleeding phenotypes (loss of < or > than 20% of estimated blood volume, calculated as 70 ml/kg for BMI<29.9, 60 ml/kg for BMI=30–39.9, and 50 ml/kg for BMI>40. Statistical elaborations highlighted a core set of proteins showing significant correlations to either total blood loss or TEG R/MA parameters.

Conclusion and clinical relevance—Though prospective verification and validation in larger cohorts will be necessary, this report suggests a potential for targeted quantitative proteomics of pre-operative plasma protein concentrations in the prediction of estimated blood loss following CPB.

Keywords

targeted proteomics; cardiopulmonary bypass surgery; medical proteomics; coagulation cascade; bleeding outcomes

1. Introduction

Cardiac surgery carries a moderate risk for extensive blood loss, with approximately 5–7% of patients producing more than 2 liters of chest tube drainage during the first 24 postoperative hours[1–4]. Peri-operatively, cardiac surgery utilizes approximately 15%–20% of the blood supply worldwide, and presents an ongoing challenge to clinicians [1,4–9]. A safe level for acceptable blood loss has not been established, but studies have shown that as little as 1 liter of chest tube drainage in the first 24 hours is independently associated with 4 times higher mortality[2]. Additionally, several recent analyses have found that in patients without exposure to blood products, chest tube drainage itself is a predictor of mortality [6,8,10]. This suggests that bleeding causes harm through mechanisms other than blood transfusion, and further exemplifies the ongoing challenge of hemostasis following these procedures[1,2,6,10]. Blood and blood product transfusions themselves have been associated with increased surgical morbidity and mortality in a dose dependent manner [4,5,7,8,11,12].

Clinical Relevance

The introduction of thrombelastography (TEG) in the surgical setting has provided new analytical opportunities to determine coagulation phenotypes in the clinical setting. Despite these advancements, adequate technology to accurately predict bleeding phenotypes for patients undergoing cardiopulmonary bypass on the basis of non-mechanical parameters is lacking. In the past decades, mechanistic studies have helped in elucidating the main events driving coagulation cascades and identifying regulatory mediators of hemostasis and fibrinolysis. However, targeted protein assays are still missing and the association of protein profiles to bleeding phenotypes and TEG readouts have yet to be discovered. Here we employed the quantitative concatamer (QconCAT) approach to quantify 123 plasma proteins from 23 patients undergoing cardiopulmonary bypass (CPB) and sternotomy. Correlation of proteomics results with TEG parameters helped expanding on the current understanding of coagulation cascades and suggesting a (correlative) role for proteins redox enzymes and extracellular matrix remodeling proteins in bleeding of cardiopulmonary bypass patients.

Overall, this report suggests a potential for targeted quantitative proteomics of pre-operative plasma protein concentrations in the prediction of estimated blood loss following cardiopulmonary bypass. Candidate markers are identified for prospective validation in larger clinical cohorts.

An important aspect of managing excessive bleeding post-operatively is identifying at-risk patients in the pre-operative setting[13]. Patients with pre-operative renal dysfunction, liver disease, and coagulopathy are known to have an increased bleeding risk [13]. Additional risk factors include: medications, age, gender, emergency cardiac surgery, length of cardiopulmonary bypass (CPB) time, repeat sternotomy, and operating surgeon/surgical technique [2,4,5,7,8,12,13]. Though these factors may alert providers to an increased risk of bleeding, currently there is not a well validated predictor of post-operative bleeding that can guide pre-operative therapy in preparation for surgery.

Over the last two decades, thrombelastography (TEG), alone or in combination with platelet mapping (PM), has emerged as a routine point of care (POC) test to assist in transfusion product decision making. TEG enables clinicians to measure interactions between cellular and plasma components of the coagulation cascade with a turnaround time of <10–15 minutes (compared to central lab results of 30–60 minutes), making it a more practical test in guiding hemostatic therapy [5,7,8,14]. Transfusion algorithms using POC testing are more restrictive than those guided by conventional coagulation testing, and they have led to 3–4 times lower transfusion volumes [5,7,8,14]. Support for use of POC testing to predict bleeding outcomes exists in small sample sets. A 2012 study by Weitzel et al. [1] showed that PM can be used to look at individual platelet receptors in the setting of CPB, and also that decreased platelet collagen receptor activity leads to higher 24-hour chest tube output. Chowdhury et al. [11] also found PM to be predictive of operative bleeding, particularly in the setting of clopidogrel or aspirin use. Despite the evidence that POC testing helps guide transfusion decisions and offers some predictive ability for bleeding outcomes, debate continues regarding inter-laboratory reliability and reproducibility as compared to standard central lab based coagulation testing. [5,7,8,10,11,14] While POC testing does provide a real time measure of coagulation as discussed here, it does lack some specificity in the exact coagulation abnormalities, and its use as a pre-operative predictive tool is only supported in small studies. With these questions in mind, the ability to measure coagulation based protein levels may lead to a predictive bleeding profile with greater specificity than current POC testing.

Previously, we used high-resolution mass spectrometry to identify substantial differences in the proteome profiles between plasma from healthy and severe trauma patients, or in animal models of trauma/hemorrhagic shock [14,16,17]. Mass spectrometry-based proteomics is indeed a valuable tool in biomarker research in health and disease [18], such as in the case of breast cancer [19]. However, despite decades of publications in the field, proteomics has hitherto struggled to deliver biomarkers readily usable for clinical purposes [20]. Indeed, while the plasma proteome list has been continuously updated during the last decade [21,22], discovery-mode proteomics approaches have largely relied upon relative quantitation strategies, which are limited by time-consuming workflows and intrinsic

difficulties in comparing results among different laboratories. In order to overcome these hurdles, quantitative proteomics technologies have been introduced over the years [23], among which the quantitative concatamer (QconCAT) technology [24–28] represents one of the latest entries with promising translational potential. Briefly, the QconCAT technology exploits heavy labeled proteotypic peptides (peptides that are characterized by sequences unique to a specific protein isoform) as internal standards to quantify proteins of interest in a given biological matrix, such as plasma from apheresis platelet supernatants [27] or stored red blood cell supernatants [28]. Heavy labeled and endogenous light peptides can be thus monitored through targeted mass spectrometry-based approaches, such as multiple reaction monitoring (MRM) assays [29,30]. Absolute protein quantitation is achieved by calculating the ratios of heavy (exogenous)/light (endogenous) peptide signals. Here we performed an observational pilot study designed to identify potential protein biomarkers of post-operative bleeding in patients undergoing primary sternotomy and cardio-pulmonary bypass (CPB). We hypothesized that pre-surgery plasma protein levels are associated with bleeding outcomes, and that quantitative proteomics may be possibly used clinically in conjunction with TEG and PM to identify unique coagulation profiles and predict patients at risk of high bleeding before invasive surgery.

2. Materials and Methods

2.1 Patient population and enrollment criteria

From 10/2013 to 5/2014, 23 patients undergoing primary sternotomy and CBP were recruited for the study (Supplementary Table 1). This protocol was approved by the Colorado Multiple Institutional Review Board (COMIRB), and written informed consent was obtained from each of the 23 patients. Exclusion criteria were: known bleeding disorders; patients on anti-platelet agents such as clopidogrel within 10 days of surgery; and patients unwilling or unable to provide written informed consent, administration of direct thrombin inhibitors; and re-sternotomy. Patients on aspirin were included. The 24-hour chest tube output was the outcome measure for postoperative blood loss. The patients were representative of cardiac surgery patients at a tertiary care academic medical center and underwent the following procedures by multiple different surgeons: valve replacement (10) coronary artery bypass grafting (8), valve replacement and coronary artery bypass grafting (2), ascending aortic aneurysm repair (1), Valsalva aneurysm repair (1), and aortic valve replacement, mitral valve anuloplasty and ventricular septal myectomy (1). None of the patients required re-exploration for surgical bleeding.

2.2 Sample Collection

Baseline blood samples for both TEG and TEG/PM were taken from the arterial cannula before heparinization and CPB. Heparin dose was 350 units/kg, with additional heparin given as needed to achieve an activated clotting time greater than 480 s, which was maintained throughout CPB. Aminocaproic acid was given as an anti-fibrinolytic agent (5g intravenous loading dose followed by 2 g/h infusion during CPB). Moderate hypothermia was employed at the discretion of the surgeon and measured by the bladder temperature (range 28–32 °C). Pump flow on CPB was adjusted to maintain a mean arterial pressure of 60 mmHg. A cell saver was employed on all patients. Transfusions of platelet and fresh

frozen plasma (FFP) were based on standard TEG results following protamine reversal and based on a published TEG-based transfusion algorithm[5].

2.3 Thromboelastography

Thromboelastography and platelet mapping were performed as previously described by Weitzel et al [1], according to the manufacturer's guidelines and previous studies [5,31,32] using PM kits (Haemoscope Corp, Niles, IL, USA) and collagen solution (1 mg/mL; Helena Lab Corp, Beaumont, TX, USA). The standard TEG reaction used 340 μ L kaolin-treated blood from a sodium citrate tube, which was transferred to a cup containing 20 μ L of 0.2 M calcium chloride and assayed in the TEG analyzer to measure thrombin-induced platelet strength ($MA_{thrombin}$). A volume of 340 μ L of heparinized blood was used for the other samples to eliminate the effects of thrombin and platelet activation. The addition of 10 μ L reptilase and 10 factor XIIIa (activator F) yielded the tensile strength of the fibrin clot (MA_{fibrin}). The remaining samples were used to determine the strength of the platelet-fibrin clot following activation by collagen, ADP or arachidonic acid (AA), labeled as $MA_{activator}$. These were achieved by combining 340 μ L of heparinized blood with 10 μ L activator R and 10 μ L platelet activator.

2.4 Protein selection

A total of 147 proteotypic peptides (accounting for 123 different proteins – multiple peptides were chosen for proteins relevant to clotting cascades) were selected based on results of previous non-targeted experiments of plasma and lymph from trauma patients and animal models [16,40,41]. In addition, we utilized publicly accessible databases, including PeptideAtlas [33] (www.peptideatlas.org), SRM Atlas [34] (www.srmatlas.org) and Global Proteome Database (<http://gpmdb.thegpm.org/>).

2.5 Proteomics

Targeted proteomics analyses were performed as previously reported [16] (Figure 1), with minor modifications as described below.

2.6 QconCAT design and expression

QconCAT constructs were designed to quantify 123 plasma-specific proteins, as previously described [35].

2.7 Sample preparation

Affinity Removal SystemTM column (4.6 $\times$ 50 mm) used to deplete the two most abundant proteins in plasma: albumin and IgG, was purchased from Agilent (Palo Alto, CA). Depletion and tryptic digestion were performed as extensively described [28].

Selected reaction monitoring analysis—A targeted Selected Reaction Monitoring (SRM) approach was performed using the QTRAP[®] 5500 interfaced with a capillary HPLC system (Agilent 1200, Palo Alto, Calif). 10 μ L of each sample was injected and directly loaded onto an Agilent C18 column (Zorbax SB-C18, 5 μ m 150 $\times$ 0.5 mm) with 5% ACN,

0.1% FA at 30 μ L/min for 3 min, as described [28]. SRM assay optimization and data analysis were performed with the aid of Skyline v2.2 software [36].

2.8 Statistical Analysis

The study was powered with fibrinogen as a representative protein related to bleeding. In patient undergoing cardiac surgery the average fibrinogen level is 4.2 ± 0.9 g/L (PMID: 18657083). Enrolling 23 patients provided 80% power to detect a 20% difference in fibrinogen levels at an alpha of 0.05. Post-hoc subjects were divided into two groups on the base of the estimated blood loss in 24 hours after surgery. We fixed 20% loss of estimated blood volume as a threshold and we divided the patients accordingly into LOW (L, average blood loss 633 mL, 15 pts) and HIGH (H, average blood loss 1511 mL, 8 pts). To overcome the problem due to multiple testing, that is the probability of observing at least one significant result due to chance, the False Discovery Rate (FDR) approach was applied. The calculation of FDR was applied to the p-values obtained from T-Test and all FDR values were < 0.304 thus indicating that the analyses performed are robust for the sake of a pilot study.

Heat maps, partial least square-discriminant analysis (PLS-DA), box and whisker plots, receiver operating characteristic (ROC) curves (of log normalized data) were calculated and plotted with the software Metaboanalyst 3.0 (<http://www.metaboanalyst.ca/MetaboAnalyst/>). Additional Spearman's correlation analyses were performed and plotted with the software GraphPad Prism (GraphPad Software Inc, La Jolla, CA, USA).

3. Results

A total of 23 patients were enrolled in this study (14 men and 9 women), with a mean blood loss of 938.5 ± 565.4 mL within 24h from CBP (Supplementary Table 1). A total of 123 proteins were investigated by monitoring 147 unique proteotypic reporter peptides and at least three transition fingerprints for each peptide (protein names, proteotypic peptide sequences, and raw values are reported in Supplementary Table 1–2). Correlations between 24-hour bleeding totals and pre- and post- surgery TEG parameters were estimated using Spearman's correlation coefficients (Supplementary Table 2), which were thus plotted upon hierarchical clustering (1-Spearman correlation – Supplementary Figure 1). Bleeding totals were collected on all 23 patients, pre-surgery TEG parameters were available on 22 patients, and post-surgery TEG parameters were available on 20 patients. Bleeding totals were negatively correlated with pre-surgery TEG parameters R and MA ($\rho_{Bleeding, TEG R} = -0.37$, $p=0.0874$, and $\rho_{Bleeding, TEG MA} = -0.31$, $p=0.16$), as well as post-surgery TEG MA ($\rho_{Bleeding, TEG MA} = -0.45$, $p=0.0466$). There was essentially no correlation between bleeding totals and post-surgery TEG R ($\rho_{Bleeding, TEG R} = -0.03$, $p=0.8998$ – Supplementary Table 2). Partial least squares regression analysis was used to characterize the overall proteomics samples with respect to estimated blood loss (EBL – total blood loss at 24h as a percentage of estimated blood volume). As an internal control for the quantitative analysis performed here, absolute concentrations for fibrinogen chains alpha, beta and gamma (FGA, FGB and FGG) – which are known to occur in triple dimers in mature fibrinogen chains-

significantly correlated among each other in all the tested samples ($p < 0.0001$ Spearman; $r > 0.94$ – Supplementary Figure 2).

The heat map and 3D-plot shown in Figure 1.A and B illustrate the results of the hierarchical clustering and PLS-DA, respectively. Clear separation of samples in the low bleeders from the high bleeders was evident in both the analyses (top 25 variables are shown in Figure 1.A). Overall, a total of 79 significant variables associated with bleeding phenotype were identified (51 upon FDR correction – Figure 1.C). Proteins (loading variables from the PLS-DA analysis) were thus sorted on the basis of VIP scores, as plotted in Supplementary Figure 3 (left panel). Top loading variables with the lowest p-values ($p < 0.05$, T-Test) are thus represented as box and whisker plots in Supplementary Figure 3, including plasminogen (PLG), fibronectin (FN1), plasminogen activator inhibitor – PAI1 (SERPINE1), FGA, FGB, FGG and coagulation factor 12 (F12).

ROC curves were thus determined to identify potential candidate protein biomarkers of bleeding phenotypes. Since ratios between two protein concentrations may carry more information than the two corresponding protein concentrations alone, we computed ratios of all possible protein pairs and chose top ranked ratios (based on p-values) to be included in the data output for further candidate biomarker analysis. Despite the potential for data overfitting, the preliminary “discovery-phase” nature of this study prompted us to include this analysis in our experimental workflow in order to increase the chance of biomarker discovery, while prospective validation in independent cohorts will be pursued in follow up studies. As a result, we could identify significant association of SERPINE1, F12, coagulation factor 13 (F13), glutathione peroxidase 3 (GPX3), transgelin (TAGLN), alpha-2-HS-glycoprotein (AHSG), fatty acid binding protein 1 (FABP1), selenium binding protein 1 (SELENBP1), vitamin D-binding protein (GC) and combined ratios (as detailed in Figure 2) with bleeding phenotypes.

Since TEG parameters associate with clot formation rapidity (R, K, α angle) and strength (MA) were determined prior to and after surgery, ROC curves were calculated and indicated that increases in MA (pre-operative, post-operative and post-protamine administration) were predictive of, and significantly correlated with lower bleeding in the studied cohort (Supplementary Figure 4), as expected. Unexpectedly, negative correlation trends for components of the fibrin clot stability and fibrinolysis cascade were observed, but did not reach significance (Supplementary Figure 4), likely due to the small sample size of this study. On the other hand, other plasma proteins that are known to play a functional role in clotting cascades – such as kininogen 1 (KNG1), PROS1, F12, SERPINE1, Inter-alpha-trypsin inhibitor heavy chain H1 (ITIH1) and kallikrein B (KLKB1) – were characterized by significant correlations ($p < 0.05$ Spearman) with EBL (Figure 3). Interestingly, proteins associated with redox homeostasis (peroxiredoxin 1 – PRDX1, selenium binding protein 1 – SELENBP1 and GPX3), platelet granule release (vimentin – VIM, retinol-binding protein 4 – RBP4 and TAGLN) and extracellular-matrix (ECM) remodeling proteins (metalloproteinase inhibitor 1 – TIMP1, matrix metalloproteinases 2 and 9 – MMP2 and MMP9) showed either significant or trending correlations with bleeding phenotypes (Figure 4).

4. Discussion

Despite recent improvements in point of care (POC) testing using devices such as TEG and TEG/PM [15], non-mechanical approaches based upon direct assessment of molecular profiles as early predictors of bleeding and coagulopathies are still missing. Here we show results from a pilot study exploiting targeted plasma proteomics profiles to predict total bleeding in CBP patients. Our results indicate that patients with similar bleeding values tend to group together, and that there are identifiable proteins significantly correlated with bleeding totals and pre-operative TEG parameters, namely R and MA, indicative of the time necessary to detect the first evidence of a clot and clot strength, respectively. The QconCAT approach herein described enables absolute quantitation of hundreds of proteins in a given sample. Absolute quantitation is relevant in that it allows inter-laboratory comparability of the data and paves the way for larger prospective validation of the candidate biomarkers identified in this pilot study and, in the long term, potential clinical translation.

Most of the proteins herein significantly associated with EBL and TEG R values, or negatively associated with TEG MA (indicative of impaired hemostasis) have been previously associated with clot formation, strength and fibrinolysis (Figure 5). Of note, almost all of them have links to known coagulation and inflammatory pathways that warrant future mechanistic investigation if validated in a larger patient cohort. Positive regulators of coagulation cascades and clot stability include fibrin/fibrinogen components, fibronectin, and F12 [37]. Other positive regulators of clot stability were significantly associated with bleeding and TEG parameters, including the tissue plasminogen activator inhibitor 1 (PAI1 – SERPINE1), a protein that has recently attracted substantial attention in the field of trauma-induced coagulopathy owing to its role in blocking plasminogen activation and inhibiting the initiation of fibrinolytic cascades [38].

Kallikrein [39] is a well-established pro-fibrinolytic agent, ultimately mediating plasminogen conversion to plasmin. Other proteins, such as matrix metalloproteinases 2 and 9 and transgelin had been previously identified as potential mediators of coagulopathic and inflammatory sequelae to trauma and hemorrhage in humans and animal models through remodeling of the extracellular matrix [40,41]. Bleeding-induced hemolysis and oxidative stress was mirrored by the distribution of PRDX1 and GPX3 in pre-operative samples, proteins that contribute to induce tissue factor-dependent activation of coagulation cascades in mice [42].

Limitations to this study include the sample size used and the elevated number of variables monitored. As a pilot study, this dataset of 23 patients only includes 8 patients with blood loss >20% EBV, thus conclusions here regarding clinical application are preliminary. Additional follow up studies should be aimed at expanding the sample size as well as the patient subsets examined. An additional limitation is in the type of surgical patient included; the patients in this data set were considered to have a low risk for bleeding in the setting of cardiac surgery. Future studies should benefit from evaluation of a higher risk bleeding group to examine if this group has distinct profiles compared to a low risk population. TEG parameters of clot formation rapidity and stability were here monitored and correlated to bleeding phenotypes and protein concentrations. However, bleeding phenotypes may have

been influenced by clot breakdown and thus future studies should be designed as to monitor parameters relevant to this end, such as LY30 and LY60 (clot lysis percentage at 30 and 60 minutes from clot formation). Finally, a second parameter of interest would be the examination of metabolite levels in combination with the proteomic profile, and addition of this could add to the predictive model.

In summary, we present a proof of principle application of mass spectrometry-based quantitative proteomics, the QconCAT technology, as a complementary tool to TEG testing for the prediction of bleeding phenotypes (high vs low bleeders) in a small cohort of patients undergoing CBP. Despite the exploratory nature of this pilot study and the evident necessity to prospectively validate the identified markers on larger cohorts, this is the first report suggesting a potential for targeted quantitative proteomics approaches in the prediction of total bleeding following CBP through the use of pre-operative concentrations of a subset of plasma proteins. Since this technology enables absolute protein quantitation, it allows for inter-laboratory data exchange and paves the way for the design of more easily implementable tests (e.g. ELISAs or other antibody-based approaches) that could inform clinicians about a patient's bleeding risk. Overall this study demonstrates that quantitative proteomics can be used to measure plasma proteins to address immediate clinically-relevant questions and complement currently used POC testing. Advances in cardiac surgery consistently produce the need to take care of patients with elevated surgical risk. The opportunity to assess the bleeding risk of a patient in the pre-operative time period in an accurate fashion would improve the ability of surgeons and anesthesiologists to prepare patients for these high risk surgeries. Resuscitative strategies might be thus tailored in a personalized patient-specific fashion, as to reduce the overall number of transfused blood-derived therapeutics, thus significantly cutting transfusion-related costs and potential risks for the patients [43].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Weitzel NS, Weitzel LB, Epperson LE, Karimpour-Ford A, et al. Platelet mapping as part of modified thromboelastography (TEG®) in patients undergoing cardiac surgery and cardiopulmonary bypass. *Anaesthesia*. 2012; 67:1158–1165. [PubMed: 22809250]
2. Dixon B, Reid D, Collins M, Newcomb AE, et al. The operating surgeon is an independent predictor of chest tube drainage following cardiac surgery. *J Cardiothorac Vasc Anesth*. 2014; 28:242–246. [PubMed: 24439890]
3. Vanek T, Straka Z, Hrabak J, Jares M, et al. Use of recombinant activated factor VII in cardiac surgery for an effective treatment of severe intractable bleeding. *Jpn Heart J*. 2004; 45:855–860. [PubMed: 15557726]

4. Despotis GJ, Filos KS, Zoys TN, Hogue CW, et al. Factors associated with excessive postoperative blood loss and hemostatic transfusion requirements: a multivariate analysis in cardiac surgical patients. *Anesth Analg*. 1996; 82:13–21. [PubMed: 8712388]
5. Shore-Lesserson L, Manspeizer HE, DePerio M, Francis S, et al. Thromboelastography-guided transfusion algorithm reduces transfusions in complex cardiac surgery. *Anesth Analg*. 1999; 88:312–319. [PubMed: 9972747]
6. Karkouti K, Wijeyesundera DN, Yau TM, Beattie WS, et al. The independent association of massive blood loss with mortality in cardiac surgery. *Transfusion (Paris)*. 2004; 44:1453–1462.
7. Bolliger D, Tanaka KA. Roles of thrombelastography and thromboelastometry for patient blood management in cardiac surgery. *Transfus Med Rev*. 2013; 27:213–220. [PubMed: 24075802]
8. Görlinger K, Shore-Lesserson L, Dirkmann D, Hanke AA, et al. Management of hemorrhage in cardiothoracic surgery. *J Cardiothorac Vasc Anesth*. 2013; 27:S20–34. [PubMed: 23910533]
9. Blood Conservation Guidelines | STS. n.d
10. Dixon B, Santamaria JD, Reid D, Collins M, et al. The association of blood transfusion with mortality after cardiac surgery: cause or confounding? (CME). *Transfusion (Paris)*. 2013; 53:19–27.
11. Chowdhury M, Shore-Lesserson L, Mais AM, Leyvi G. Thromboelastograph with Platelet Mapping(TM) predicts postoperative chest tube drainage in patients undergoing coronary artery bypass grafting. *J Cardiothorac Vasc Anesth*. 2014; 28:217–223. [PubMed: 24630471]
12. Weitzel NS. Point of Care Testing and Cardiac Surgery 2014. *J Cardiothorac Vasc Anesth*. 2014; 28:207–209. [PubMed: 24630469]
13. Christensen MC, Krapf S, Kempel A, von Heymann C. Costs of excessive postoperative hemorrhage in cardiac surgery. *J Thorac Cardiovasc Surg*. 2009; 138:687–693. [PubMed: 19698857]
14. Lee L, Moore EE, Hansen KC, Silliman CC, et al. It's not your grandfather's field plasma. *Surgery*. 2013; 153:857–860. [PubMed: 23122899]
15. Karon BS. Why is everyone so excited about thromboelastography (TEG)? *Clin Chim Acta Int J Clin Chem*. 2014; 436:143–148.
16. Dzieciatkowska M, Silliman CC, Moore EE, Kelher MR, et al. Proteomic analysis of the supernatant of red blood cell units: the effects of storage and leucoreduction. *Vox Sang*. 2013; 105:210–218. [PubMed: 23663258]
17. D'Alessandro A, Dzieciatkowska M, Peltz ED, Moore EE, et al. Dynamic Changes in Rat Mesenteric Lymph Proteins Following Trauma Using Label-Free Mass Spectrometry. *Shock Augusta Ga*. 2014
18. Meng Z, Veenstra TD. Proteomic analysis of serum, plasma, and lymph for the identification of biomarkers. *Proteomics Clin Appl*. 2007; 1:747–757. [PubMed: 21136731]
19. Zhang F, Chen JY. Discovery of pathway biomarkers from coupled proteomics and systems biology methods. *BMC Genomics*. 2010; 11(Suppl 2):S12.
20. Anderson NL, Anderson NG. The human plasma proteome: history, character, and diagnostic prospects. *Mol Cell Proteomics MCP*. 2002; 1:845–867. [PubMed: 12488461]
21. Omenn GS, States DJ, Adamski M, Blackwell TW, et al. Overview of the HUPO Plasma Proteome Project: results from the pilot phase with 35 collaborating laboratories and multiple analytical groups, generating a core dataset of 3020 proteins and a publicly-available database. *Proteomics*. 2005; 5:3226–3245. [PubMed: 16104056]
22. Omenn GS, Aebersold R, Paik YK. 7(th) HUPO World Congress of Proteomics: launching the second phase of the HUPO Plasma Proteome Project (PPP-2) 16–20 August 2008, Amsterdam, The Netherlands. *Proteomics*. 2009; 9:4–6. [PubMed: 19053147]
23. Bachi A, Bonaldi T. Quantitative proteomics as a new piece of the systems biology puzzle. *J Proteomics*. 2008; 71:357–367. [PubMed: 18640294]
24. Beynon RJ, Doherty MK, Pratt JM, Gaskell SJ. Multiplexed absolute quantification in proteomics using artificial QCAT proteins of concatenated signature peptides. *Nat Methods*. 2005; 2:587–589. [PubMed: 16094383]

25. Pratt JM, Simpson DM, Doherty MK, Rivers J, et al. Multiplexed absolute quantification for proteomics using concatenated signature peptides encoded by QconCAT genes. *Nat Protoc.* 2006; 1:1029–1043. [PubMed: 17406340]
26. Russell MR, Achour B, McKenzie EA, Lopez R, et al. Alternative Fusion Protein Strategies to Express Recalcitrant QconCAT Proteins for Quantitative Proteomics of Human Drug Metabolizing Enzymes and Transporters. *J Proteome Res.* 2013; 12:5934–5942. [PubMed: 24124648]
27. Dzieciatkowska M, D'Alessandro A, Burke TA, Kelher MR, et al. Proteomics of apheresis platelet supernatants during routine storage: Gender-related differences. *J Proteomics.* 2015; 112:190–209. [PubMed: 25201077]
28. D'Alessandro A, Dzieciatkowska M, Hill RC, Hansen KC. Supernatant protein biomarkers of red blood cell storage hemolysis as determined through an absolute quantification proteomics technology. *Transfusion (Paris).* 2016
29. Steen H, Mann M. The ABC's (and XYZ's) of peptide sequencing. *Nat Rev Mol Cell Biol.* 2004; 5:699–711. [PubMed: 15340378]
30. Marx V. Targeted proteomics. *Nat Methods.* 2013; 10:19–22. [PubMed: 23547293]
31. Bliden KP, DiChiara J, Tantry US, Bassi AK, et al. Increased risk in patients with high platelet aggregation receiving chronic clopidogrel therapy undergoing percutaneous coronary intervention: is the current antiplatelet therapy adequate? *J Am Coll Cardiol.* 2007; 49:657–666. [PubMed: 17291930]
32. Craft RM, Chavez JJ, Bresee SJ, Wortham DC, et al. A novel modification of the Thrombelastograph assay, isolating platelet function, correlates with optical platelet aggregation. *J Lab Clin Med.* 2004; 143:301–309. [PubMed: 15122174]
33. Desiere F, Deutsch EW, King NL, Nesvizhskii AI, et al. The PeptideAtlas project. *Nucleic Acids Res.* 2006; 34:D655–D658. [PubMed: 16381952]
34. Picotti P, Lam H, Campbell D, Deutsch E, et al. A database of validated assays for the targeted mass spectrometric analysis of the *S. cerevisiae* proteome. *Nat Methods.* 2008; 5:913–914. [PubMed: 18974732]
35. Matic I, Jaffray EG, Oxenham SK, Groves MJ, et al. Absolute SILAC-compatible expression strain allows Sumo-2 copy number determination in clinical samples. *J Proteome Res.* 2011; 10:4869–4875. [PubMed: 21830832]
36. MacLean B, Tomazela DM, Shulman N, Chambers M, et al. Skyline: an open source document editor for creating and analyzing targeted proteomics experiments. *Bioinforma Oxf Engl.* 2010; 26:966–968.
37. Hahn LHE, Yamada KM. Isolation and biological characterization of active fragments of the adhesive glycoprotein fibronectin. *Cell.* 1979; 18:1043–1051. [PubMed: 519764]
38. Chapman MP, Moore EE, Moore HB, Gonzalez E, et al. Overwhelming tPA release, not PAI-1 degradation, is responsible for hyperfibrinolysis in severely injured trauma patients. *J Trauma Acute Care Surg.* 2016; 80:16-23-25. [PubMed: 26491796]
39. Bork K, Kleist R, Hardt J, Witzke G. Kallikrein-kinin system and fibrinolysis in hereditary angioedema due to factor XII gene mutation Thr309Lys. *Blood Coagul Fibrinolysis Int J Haemost Thromb.* 2009; 20:325–332.
40. D'Alessandro A, Dzieciatkowska M, Peltz ED, Moore EE, et al. Dynamic changes in rat mesenteric lymph proteins following trauma using label-free mass spectrometry. *Shock Augusta Ga.* 2014; 42:509–517.
41. Dzieciatkowska M, D'Alessandro A, Moore EE, Wohlaue M, et al. Lymph is not a plasma ultrafiltrate: a proteomic analysis of injured patients. *Shock Augusta Ga.* 2014; 42:485–498.
42. Sparkenbaugh EM, Chanrathammachart P, Wang S, Jonas W, et al. Excess of heme induces tissue factor-dependent activation of coagulation in mice. *Haematologica.* 2015
43. D'Alessandro A, Kriebardis AG, Rinalducci S, Antonelou MH, et al. An update on red blood cell storage lesions, as gleaned through biochemistry and omics technologies. *Transfusion (Paris).* 2015; 55:205–219.
44. Kollman JM, Pandi L, Sawaya MR, Riley M, Doolittle RF. Crystal structure of human fibrinogen. *Biochemistry (Mosc).* 2009; 48:3877–3886.

Figure 1.

A. Hierarchical clustering analysis was performed on protein levels from 123 proteins collected on 23 subjects. **B.** Partial least squares discriminant analysis (PLS-DA) of Low (<20% estimated blood loss, EBL) and High (>20% EBL) subjects on the basis of protein concentration phenotypes. **C.** Significance analysis of microarrays (SAM) plot indicating the total number of proteins showing a statistically significant difference ($p < 0.05$) between the two groups (Low vs High), with and without false discovery rate (FDR) correction.

Figure 2.

Receiver operating characteristic (ROC) curves illustrates the performance of protein ratios to discriminate between bleeding phenotypes. Areas under the curve (AUC) are indicated for each entry where the true positive rate (TPR) is plotted against the false positive rate (FPR) at various threshold settings.

Figure 3.

Correlation curves showing significant association between clotting cascade and stability components and estimated blood loss (EBL) (green: low bleeders; red: high bleeders).

Figure 4.

Pre-operative plasma concentrations of proteins associated with redox homeostasis, granule release, extracellular matrix (ECM) remodeling showed unanticipated significant correlations with bleeding phenotypes (green: low bleeders; red: high bleeders).

Figure 5. A summary of clotting cascades and fibrinolysis protein components monitored in this study. In yellow and black, proteins showing significant negative or positive correlation to estimated blood loss, respectively, as determined in this pilot study.