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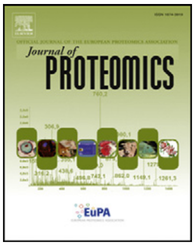

Technical note

Plasma QconCATs reveal a gender-specific proteomic signature in apheresis platelet plasma supernatants

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ABSTRACT

Clinical translation of proteomic technologies is often hampered by technical limitations, including inter-laboratory inconsistencies of label-free derived relative quantification, time-consuming analytical approaches and the subsequent challenge of performing proteomic analyses on large cohorts of subjects. Here we introduce plasma QconCAT-based targeted proteomics, an approach that allows the simultaneous absolute quantitation down to the picogram level of hundreds of proteins in a single liquid chromatography-selected reaction monitoring mass spectrometry run. We demonstrate the robustness of the approach by analyzing apheresis platelet concentrate supernatants at storage day 1 and the end of the shelf life for this blood-derived therapeutic, day 5. The targeted approach was repeatable and robust revealing potential gender-specific signatures across a set of three male and female donors. This technical note represents a proof-of-principle of the application of QconCAT-based MRM strategies to transfusion-medicine relevant issues, such as storage and gender-dependent proteomic signatures in blood-derived therapeutics.

Biological significance

Gender differences in the proteome composition of apheresis platelet supernatants have always been postulated, and might underlie a higher risk of adverse reactions when transfusing apheresis products from female donors. Preliminary proteomic studies provided an overview of gender-dependent relative compositional differences in the proteome of apheresis platelet supernatants during routine storage in the blood bank. Here we apply a proteomics approach for absolute quantitation of approximately 100 proteins in apheresis platelet supernatants from male and female donors at storage days 1 and 5. Absolute quantitative proteomic analyses allowed us to confirm and expand on previous observations about gender and storage-dependency of platelet supernatant protein profiles.

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Platelet proteomics is an important research endeavor [1] that holds immediate translational potential in the fields of transfusion medicine [2–4], immunology [5], and clinical biology [6]. Transfusion of apheresis platelet concentrates represents a key component of supportive care for thrombocytopenic patients. Even though apheresis platelet concentrates can be stored up to 5–7 days in most countries, the quality of platelets is affected by storage duration, resulting in partially compromised cell morphology, hypotonic shock responses, altered cell volume, density, activation and coagulant activity [2–4]. Early studies suggested that platelet storability might be affected by donor gender as well [7]. Gender differences in donated apheresis platelets include a higher risk of anti-HLA or anti-neutrophil antibodies in plasma from parous women, which could result in adverse transfusion reactions such as transfusion-related acute lung injury (TRALI) [7]. Recent proteomic evidence has been provided about the apparent increase in the levels of pro-inflammatory factors and activation markers in the supernatants of apheresis platelets from female donors during routine storage in the blood bank. The accumulation of these factors in apheresis platelet supernatants might further increase the risk of TRALI associated with transfusion of platelet concentrates from female donors [8]. In this view, it is worth noting that, in the last few years, the supernatants of platelets concentrated for transfusion purposes have emerged as key indicators of storage quality [4,8–10]. Unfortunately, these key translational studies are compromised by the limited capacity to perform proteomic analyses on large cohorts of subjects. Indeed, these studies often rely upon time-consuming workflows characterized by a long series of time consuming steps; protein extraction, solubilization, digestion, and separation via long nano-high performance liquid chromatography–tandem mass spectrometry — HPLC–MS/MS runs. In addition, most proteomic studies performed to date are based upon relative quantitative approaches, which hamper inter-laboratory comparability of the results. To cope with these inconveniences, we propose the application of a targeted, micro-LC-selected reaction monitoring mass spectrometry (SRM) approach. This approach is based upon the absolute quantitation of trypsin-digested protein mixtures against spiked in ^{13}C-labeled quantitative concatenamer (QconCAT) derived internal standards [11]. So far, the QconCAT method has been applied to a limited number of clinically relevant issues [12,13], basically owing to the technical difficulties associated with the selection of adequate proteotypic peptides and the generation of QconCAT constructs. Here we show its application to plasma supernatants from apheresis platelet concentrates for transfusion purposes, and demonstrate the potential of this technology to monitor storage and gender-dependency of proteomic changes in apheresis platelet supernatants during routine storage in the blood. The present results might inform policies regarding gender issues associated with apheresis platelet storability and safety.

Our results are consistent with recent untargeted label-free proteomics analyses of plasma [14] and platelets [8] from our lab. At the same time, the reported results expand upon recent literature by providing absolute quantitation values for each detected protein. Though the technology is not novel, here we show for the first time its application to a transfusion medicine-relevant issue and the potential for its implementation in routine proteomics and possibly clinical analysis of plasma-related matrices (such as supernatants of blood-derived therapeutics).

Briefly, 6 healthy donors (3 females and 3 males) donated one unit of apheresis platelets using a Cobe Trima apparatus with appropriate leukoreduction ($<5 \times 10^6$/unit, consistent with AABB guidelines and the literature [8]). Samples were collected on storage days 1 and 5. Platelet supernatants were obtained through serial centrifugation, aliquoted and stored at −80 °C [8]. Before quantitative proteomics analysis, depletion of the top 2 most abundant plasma proteins (Serum Albumin & IgG) was performed by Multiple Affinity Removal System™ columns (4.6 × 100 mm, Agilent — Palo Alto, CA), as previously reported [8].

Four QconCAT constructs (Supplementary table 1) were designed to quantify plasma and platelet specific proteins previously found in healthy individuals, and various trauma patients. Proteotypic peptides were selected from previous experimental data (non-targeted plasma proteomics [14]) and publicly accessible databases, including PeptideAtlas [15] SRM Atlas [16] and Global Proteome Database [17]. Criteria for proteotypic peptide selection and optimal QconCAT designing were consistent with the literature [18]. The resulting list of 139 unique peptides is provided in Supplementary table 1. QconCAT DNA constructs were synthesized de novo by Genscript (Piscataway, NJ) and cloned into pET21b. *Escherichia coli* auxotrophic strain BL21(l)DE3-LysA ArgA [19] was transformed with the plasmid and cultured in minimal medium supplemented either with unlabeled or $^{13}C_6$ arginine and $^{13}C_6$ lysine at 0.1 mg/ml (Sigma Aldrich). The cells were grown to mid-log phase (A600 0.6–0.8), at which point expression was induced by adding 1 mM isopropyl-D-1-thiogalactopyranoside. After 4 h of growth at 37 °C the cells were harvested by centrifugation and processed as previously described with minor modifications [11]. Briefly, the cells were lysed with the BugBuster Protein Extraction Reagent (EMD Millipore). Inclusion bodies were suspended in 20 mM phosphate buffer, 6 M guanidinium chloride, 0.5 M NaCl, 20 mM imidazole, pH 7.4. QconCAT proteins were purified by affinity chromatography using a nickel-based resin. The purified QconCAT was desalted by three rounds of dialysis against 100 volumes of 10 mM ammonium bicarbonate, pH 8.5. Known concentrations of recombinant isotopically labeled QconCAT proteins were mixed with the depleted platelet supernatants. Trypsinization (FASP protocol-sequencing grade modified trypsin — Promega, through a 10 kDa molecular weight cutoff filter and centrifugation at 14,000 *g* for 15 min) was performed upon reduction (10 mM DTT in 8 M urea in 0.1 M Tris–HCl, pH 8.5, incubation for 30 min at RT) and alkylation of the samples (55 mM iodoacetamide in 8 M urea in 0.1 M Tris–HCl, pH 8.5, incubated for 30 min at RT). Six washes were then performed, with 8 M urea in 0.1 M Tris–HCl, pH 8.5 solution followed by 50 mM ammonium bicarbonate buffer. Overnight trypsin digestion at 37 °C was performed before recovering peptides from the filter using 30% ACN. Samples were then dry vacuumed to ~2 μL and reconstituted to 50 μL with 0.1% formic acid. The resultant peptide mixture was analyzed in duplicate by LC-SRM. Briefly, a targeted SRM approach was performed using the QTRAP® 5500 interfaced with a capillary HPLC system