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Plasma proteomes of acute myeloid leukemia patients treated with transfusions reveal signatures of inflammation and hemostatic dysregulation

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Abstract

Background Bone marrow aplasia is a common feature in acute myeloid leukemia (AML) patients during their remission induction treatment, and is associated with potential complications such as bleeding, infection and anemia. Frequent platelet and red cell transfusions are administered to prevent and treat these complications. However, platelet counts are poorly associated with bleeding events in this population. Therefore, plasma protein levels could add valuable insights to improve our understanding of the patient's health state. In this study, we aimed to delineate the plasma proteome, including inflammatory pathways, hemostatic and immune components, of AML patients during treatment with intensive transfusion support.

Methods We employed unbiased mass spectrometry (MS)-based proteomics on longitudinal plasma samples from 10 AML patients during intensive-transfusion treatment phase with healthy individuals as baseline control.

Results A total of 450 proteins were quantified in plasma samples from AML patients and healthy controls. Alteration in proteins levels were mainly observed for proteins involved in inflammation (e.g. SAA1 and CRP), and complement (e.g. C9 and MASP2) when comparing AML versus healthy individuals. Correlation analysis revealed additional affected protein dynamics, including proteins associated with coagulation cascade, endopeptidase inhibitors activity and lipoprotein remodeling.

Conclusion The plasma proteome from AML patients during intensive treatment shows a disbalance in inflammation, endopeptidase inhibitors activity, lipoprotein remodeling, coagulation and complement. These effects and potential associations with bleeding risk will be further studied in a bigger cohort.

Keywords Transfusion, AML, Proteomics, Plasma profiling, Platelets, Red blood cells, Chemotherapy

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Background

Acute myeloid leukemia (AML) is a common haemato-oncological disease [1] affecting 4.3 out of 100,000 individuals annually [2] with an increasing incidence rate over the last two decades [3, 4]. For the majority of AML patients, the first treatment steps include a course of remission induction chemotherapy [1, 5]. This results in temporary bone marrow aplasia leading to low counts of red blood cells (RBC), platelets (PLT) and neutrophils [6, 7], which are associated with a high risk of adverse events including anemia [8], bleeding events [9, 10] and infections [11]. To treat and prevent these complications, PLT counts, and hemoglobin (Hb) levels are closely monitored in patients. In general, PLT or RBC transfusions are administered based on specific thresholds of PLT counts or Hb levels, as well as individual risk factors [12]. However, PLT counts and Hb levels do not necessarily associate with the patient's risk [13–15], which may lead to unnecessary transfusions. More importantly, transfusions may have detrimental effects on the patients as they are associated with febrile and allergic reactions, as well as transfusion-related acute lung injury (TRALI) and transfusion-associated circulatory overload (TACO) [16]. Furthermore, PLT transfusions may lead to alloimmunization and associated PLT refractoriness [17, 18].

Characterization of the underlying contributors to the overall state of AML patients during their transfusion-dependent treatment period could provide insight into additional affected processes that might contribute to the risk of anemia, bleeding and infections. Combining signatures of circulating protein levels with clinical data may enable this, as the plasma proteome has shown tremendous potential in reflecting the health state of individuals demonstrated by previous mass spectrometry (MS) based studies [19–23]. Moreover, proteomics studies in AML patients have shown the potential of this technology by investigating disease landscape and heterogeneity [24, 25] as well as changes after induction chemotherapy [26]. Additionally, previous work from our group has identified an increase of acute phase proteins in AML patients undergoing febrile episodes [27].

Despite its potential, this technology has not been applied to understand responses to transfusions supportive care in AML patients. Hence, in an effort to gain a deeper understanding of the overall plasma proteome during the intensive transfusion period of these patients compared to healthy controls, we applied MS-based plasma profiling. To limit previously described factors that may affect the plasma proteomes, we evaluated protein signatures in a homogenous group of 10 AML patients undergoing remission induction therapy.

Materials and methods

Patient cohort

Samples collected in the Pathogen Reduction Evaluation & Predictive Analytical Rating Score (PREPAReS) study (registered at NTR2106 and NCT02783313) were used for this study. The clinical design and ethical approval of the PREPAReS study has been described previously [28, 29]. This study included adult hemato-oncology patients undergoing chemotherapy-induced thrombocytopenia who required at least 2 platelet transfusions throughout their treatment period. From the 567 randomizations in the PREPAReS database, we selected randomizations from AML patients ($n=271$) undergoing a remission induction treatment phase ($n=185$). Patients were included if (I) received the control arm product (PLTs in plasma) only ($n=58$), (II) did not have an infection at the time of randomization ($n=46$) and (III) did not receive a transplant nor PLT transfusion prior to the treatment phase ($n=12$). Out of the 12 selected patients, longitudinal ethylenadiminetetraacetic acid (EDTA) plasma samples were available for 10 patients ($n=34$ samples) (Supplemental Fig. S1). Plasma was stored short-term at -30 °C and transferred to -80 °C until further analysis.

Healthy controls

Whole blood was drawn from 11 anonymous healthy individuals into EDTA tubes and centrifuged for 20 min at 120 *g* at room temperature (RT) followed by a 2-step centrifugation protocol of the obtained PLT rich plasma, 10 min at 2,000 *g* followed by 10 min at 10,000 *g* at RT to collect PLT poor plasma. Aliquots were stored at -80 °C until further analysis. Ethical approval was obtained from the Sanquin Ethical Advisory Board in accordance with Dutch regulations and the Declaration of Helsinki.

Sample preparation

Frozen plasma samples were thawed at 37 °C. Next, 10 µL of each sample was diluted 1:60 in 100 mM Tris(hydroxymethyl)aminomethane hydrochloride (Tris, Life Technologies, UK) (pH = 8.0). 5 µL reduction and alkylation buffer, consisting of 20 mM Tris(2-carboxyethyl)phosphine (Thermo Fisher Scientific, USA) and 80 mM chloroacetamide (Sigma Aldrich, Germany) in 100 mM Tris (pH = 8.0) was added to 9 µL of diluted plasma. The protein mixture was resuspended, heated at 95 °C for 5 min and cooled down to RT. The proteins were digested overnight at 25 °C using 100 ng MS-grade Trypsin Gold (Promega, USA) in 50 mM Tris (pH = 8.0). Peptides were acidified with 5 µL of trifluoroacetic acid (Thermo Fisher Scientific, USA) to a final concentration of 1% (v/v) and approximately 500 ng peptides were loaded onto EvoTip Pure tips (EvoSep, Denmark) according to manufacturer's guidelines. To evaluate the

robustness of our analytical workflow, we implemented a study-wide pooled peptide sample as quality control.

Proteomics analysis

Samples were analyzed using an Evosep One liquid chromatography system on a 15 cm C18 analytical column (Evosep Pepsep, 1.5 µm beads, 100 µm ID, EV-1137) and the pre-built 30 samples per day method (Evosep One) at a 0.5 µl/min flow rate. Peptides were dispersed into an Orbitrap Fusion™ Lumos™ Tribrid™ Mass Spectrometer (Thermo Fisher Scientific, USA) controlled with Xcalibur software 4.1 (Thermo Fisher Scientific, USA) equipped with an electrospray ionization source using data independent acquisition mode. For MS1 a scan from 390–1010 m/z at 60 K resolution was made with standard AGC and 100 ms maximal injection time, followed by 75 consecutive MS2 scans with an 8 m/z window size and 1 m/z overlap in a precursor mass range of 400–1000 m/z. Higher-energy collisional dissociation fragmentation was set to normalized collision energy of 23%. MS2 scans were made in centroid mode at 30 K resolution with standard AGC standard and 54 maximal injection time. The default charge state was set to 2.

Proteomic data processing

RAW files were processed with DIA-NN (v1.8.1) [30] using the reviewed human proteome database (Swiss-Prot Database, 20,423 entries, downloaded on 8 August 2023) using the library-free search. Standard settings were used in DIA-NN except for protein inference set to protein names from fasta, quantification mode on robust LC (high accuracy), match between runs and no shared spectra was enabled, and heuristic protein inference strategy was disabled. Data was loaded into R (v4.1.1) [31] and tidyverse [32] was used for data wrangling and visualization. The sample names of the report files generated by DIA-NN were anonymized. The protein groups were filtered to only include protein label free quantification (LFQ) intensity values based on 2 or more unique precursors per sample (Table S1B). Proteins were considered accurately quantified if they were present in at least 40% of all samples of either of the two study groups. Data were $\log_2$ transformed and missing values were imputed using a normal distribution (width = 0.3; downshift = 1.8).

Proteomic data analysis

Accuracy of the workflow was determined by calculating the coefficient of variation (CV) across all quality control samples per protein based on non-$\log_2$ transformed intensities. Principal component analysis (PCA) was performed on non-imputed data with prcomp. Moderate t-tests were performed using Limma [33] to identify statistically differential levels of proteins using block on the individuals, proteins with an absolute log fold change ≥ 1 and Benjamini–Hochberg adjusted p-value < 0.05 were considered significant. Results were visualized using Euller [34], ComplexHeatmap [35]. Global correlation analysis was performed by calculating the Pearson correlation based on imputed values for all 450 proteins with the Hmics package [36]. Protein networks were defined based on a Pearson correlation threshold of 0.8 and visualized in Cytoscape [37]. Functional enrichment analysis was performed in Cytoscape, plugin ClueGO version 2.5.10 [38]. Proteins were annotated using gene ontology terms from biological processes and molecular function. The parameters used to run ClueGo were as follows: GO tree interval 3–8, minimum number of genes per cluster was set to 3 representing a minimum of 5% from a pathway. Results were built applying gene ontology term (GOTerm) fusion to reduce redundancy of GOTerms. Only pathways with a p value < 0.05 were taken into account. Statistical and grouping parameters were set as default. Biological pathways illustrated in each cluster were defined as the top term with > 5% associated genes and lowest term corrected p-value in each GOGroup.

Data sharing statement

Anonymized processed data is available in the Supplemental Table 1B and scripts used for the proteomic analysis and visualization are available on GitHub at https://github.com/evasmit/PlasmaProteomicsPreparesPilot.

Results

Trends in clinical data during transfusion periods

In our cohort, we analyzed 34 longitudinal plasma samples from 10 patients (Fig. 1A - Table S1A) collected throughout the time when PLT and RBC transfusions were frequently administered. Overall, the transfusion window (time from inclusion until 48 h after the last transfusion) in these patients lasted 19 [15.5–25.5] days, with a median of 9 [5.5–14] PLT and 6.5 [3.7–6.2] RBC transfusions (Table 1). Over time, PLT counts and Hb levels varied amongst patients, but remained below the healthy reference values ($150–400 \times 10^9$/L for PLT and 7.1–9.5 mmol/L for Hb) throughout the studied phase (Fig. 1B - Fig. 1C – Supplemental Fig. S2). Furthermore, we examined PLT counts and Hb levels at the days of transfusions to define the trigger values applied for transfusions in these patients. PLT transfusions were administered at a median PLT count of 11 [7—29] $\times 10^9$/L (Fig. 1D), while a median of 4.7 [4.4 –4.8] Hb mmol/L (Fig. 1E) was observed for RBC transfusions.

Global plasma proteomic differences in the cohort

To evaluate the robustness of our MS-based plasma profiling, we determined the coefficient of variations

Table 1 Demographic, transfusion and laboratory information from the cohort studied. *Range = minimum and maximum value

		AML patients	Healthy controls
Sex	Female, No. (%)	3 (30%)	8 (73%)
	Male, No. (%)	7 (70%)	3 (27%)
Age	Mean (SD)	60 (7)	42 (11)
BSA		1,9 (0,26)	*NA*
Transfusion	Platelets, median (range)*	9 (3—20)	*NA*
	RBCs, median (range)*	6,5 (2—12)	*NA*
Hb (mmol/L), median (range)*		5,1 (3.97—7.30)	*NA*
Platelets 10^9cells/L, median (range)*		20 (1—668)	*NA*
While blood cells 10^9/L, median (range)*		0,5 (0—30.6)	*NA*
Samples per patient, median (range)*		3 (2—5)	1

(CVs) of 4 repeated injections of a study-wide quality control sample. This showed that 93% of protein CVs were below a threshold of 30% (Fig. 2A – Table S1C). Out of the 450 proteins quantified in this study (Table S1D), 414 proteins were quantified in AML patients and 425 in healthy controls (Fig. 2B). Further inspection showed that 389 were quantified in both groups, while 36 and 25 proteins were exclusive to controls or AML patients, respectively. Proteins only quantified in controls included coagulation factor VII (F7) which initiates the extrinsic pathway of blood coagulation, platelet factor 4 (PF4), a chemokine released by platelets upon activation, and intercellular adhesion molecule 2 (ICAM2) which is constitutively expressed on all vascular endothelial cells. In contrast, platelet proteins such as filamin-A (FLNA) and talin-1 (TLN-1) and stress response proteins hepcidin (HAMP) and ICOS ligand (ICOSLG) were only quantified in AML patients (Fig. 2C – Table S1E).

Principal component analysis (PCA) revealed that the plasma proteomes from specific patients tended to cluster closer together, suggesting individual-specific plasma profiles. Overall, we observed a segregation between samples from AML patients and controls, with patient 3, 6, and 9 clustering closer to controls compared to patients 1, 4 and 10 (Fig. 2D). Through exploration of the loading scores along principal component (PC)1, we observed that total serum amyloid protein A (SAA)-1 and 2 (SAA1;SAA2) were the most important drivers of this separation (Fig. 2E). Protein levels of SAA1;SAA2 in AML patients remained higher over time as compared to levels in controls. Despite constant increased levels of SAA1;SAA2 compared to controls (dotted line), the protein longitudinal trends varied between AML patients (Fig. 2F). In general, patients with an overall higher trend of SAA1;SAA2 throughout their treatment tended to cluster further from controls.

Plasma proteomic differences of AML patients compared to healthy controls

To determine differences in plasma protein levels of AML patients throughout their treatment period, we performed statistical analysis using controls as a reference. In total, 11 significant alterations in protein levels were found, of which 10 proteins were increased, and cathelicidin antimicrobial peptide (CAMP) was decreased in AML patients compared to healthy controls(Fig. 3A - Table S1F). Notably, 9 out of the 11 significant altered proteins in AML patients are enriched in the liver (Table S1G). In line with the PCA, we found acute phase proteins, including C-reactive protein (CRP) and SAA1;SAA2 among the significantly increased proteins. In addition, we observed increased levels of complement proteins (e.g. complement component 9 (C9) and mannan-binding lectin serine protease 2 (MASP2)).

Next, we evaluated how these proteins changed throughout the treatment phase in our patient cohort. To this end, we plotted protein levels for each individual patient over time for HAMP, CAMP, C9 and serum amyloid protein A-2 (SAA2). This showed that although a general upregulation was observed, the individual proteins revealed unique trends. Stable levels over time between and within individual patients were observed for C9, whereas individual-specific trends with dynamic changes were found for HAMP and SAA2. For example, we observed an increasing trend for HAMP and SAA2 levels towards the end of the treatment period (patient 3) and stable levels (patient 4) (Fig. 3B).

Changes in protein dynamics of AML patients during the transfusion window compared to healthy controls

We performed global protein correlation analysis to identify proteins with similar dynamics across AML patients (Fig. 4A - Table S1H). All450 proteins were correlated to

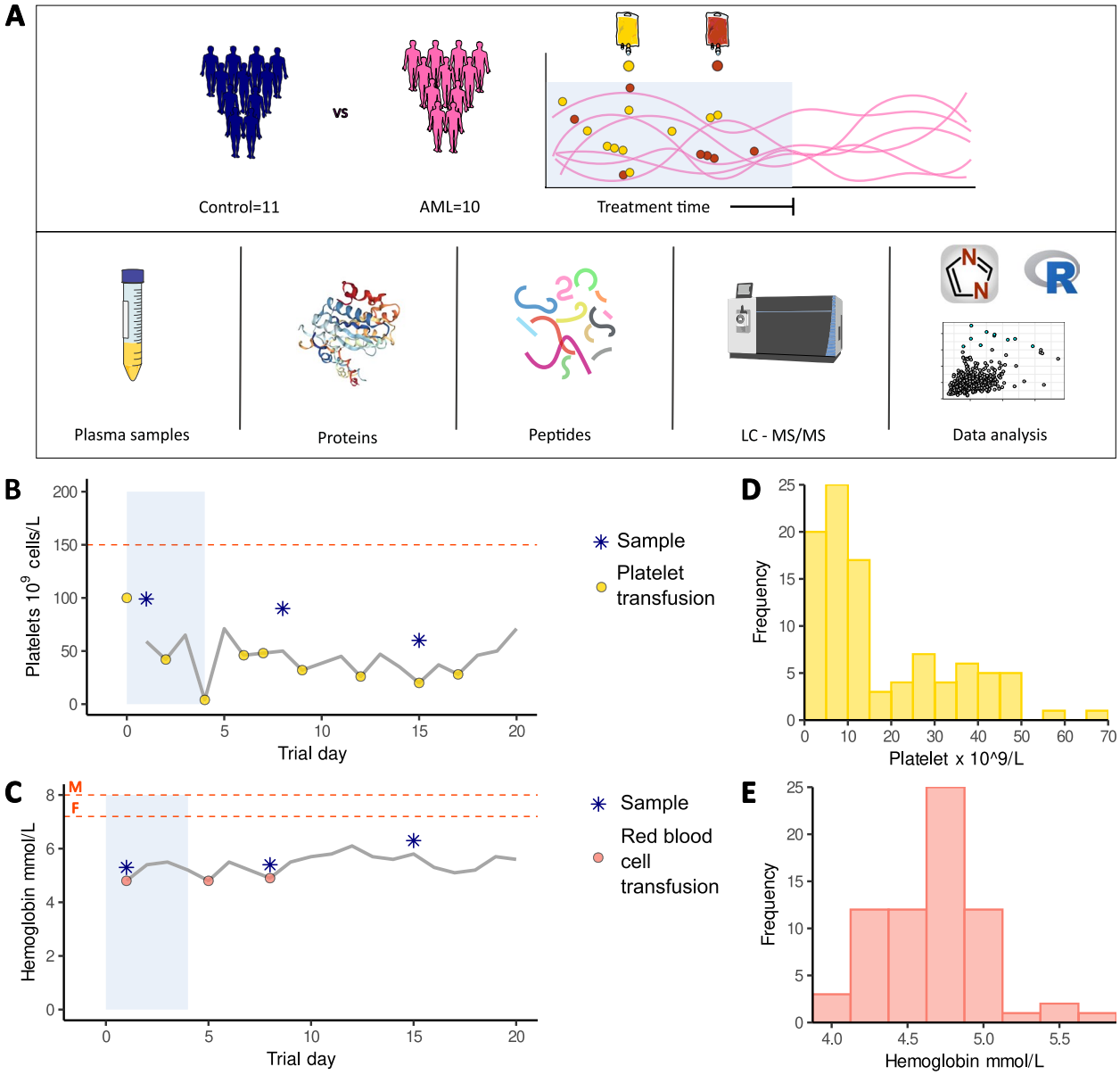

Fig. 1 Study design and trends in transfusion triggers applied for platelet- and red blood cell concentrates. **A** Schematic describing the study design. Plasma samples from healthy controls (blue, $n=11$) and AML (pink, $n=10$) were included in the study design. For the AML patients, longitudinal samples were collected during the patients' intensive transfusion support phase, during which they received both platelet (yellow dots) and red blood cells (red dots) transfusions. Plasma samples were processed for MS-based plasma profiling by tryptic digestion of plasma proteins into peptides. These peptides were then analyzed using liquid chromatography coupled to tandem mass spectrometer (LC–MS/MS). Data was processed using DIA-NN and analysis was carried out in R. **B** Example of platelet counts (PLT, $\times 10^9$/L) over time during the intensive transfusion supportive phase for one patient. Platelet minimum reference value is represented with a dashed line at 150×10^9/L. Platelet transfusions administered are shown in yellow dots and collected plasma samples are shown as blue stars. **C** Example of hemoglobin levels (Hb, mmol/L) over time during the intensive transfusion supportive phase for one patient. Hemoglobin minimum reference values are represented with dashed lines (female 7,2 and male 10,5 mmol/L). Red blood cell transfusions are shown in red dots and collected plasma samples are shown as blue stars. The shaded area represents the treatment phase studied, defined from day of randomization until 48 h after the last transfusion (either platelets or red blood cells) received. **D** Histogram depicting the transfusion thresholds recorded for platelet concentrates in this cohort of AML patients. **E** Histogram depicting the transfusion thresholds recorded for red blood cells transfusions in this cohort of AML patients

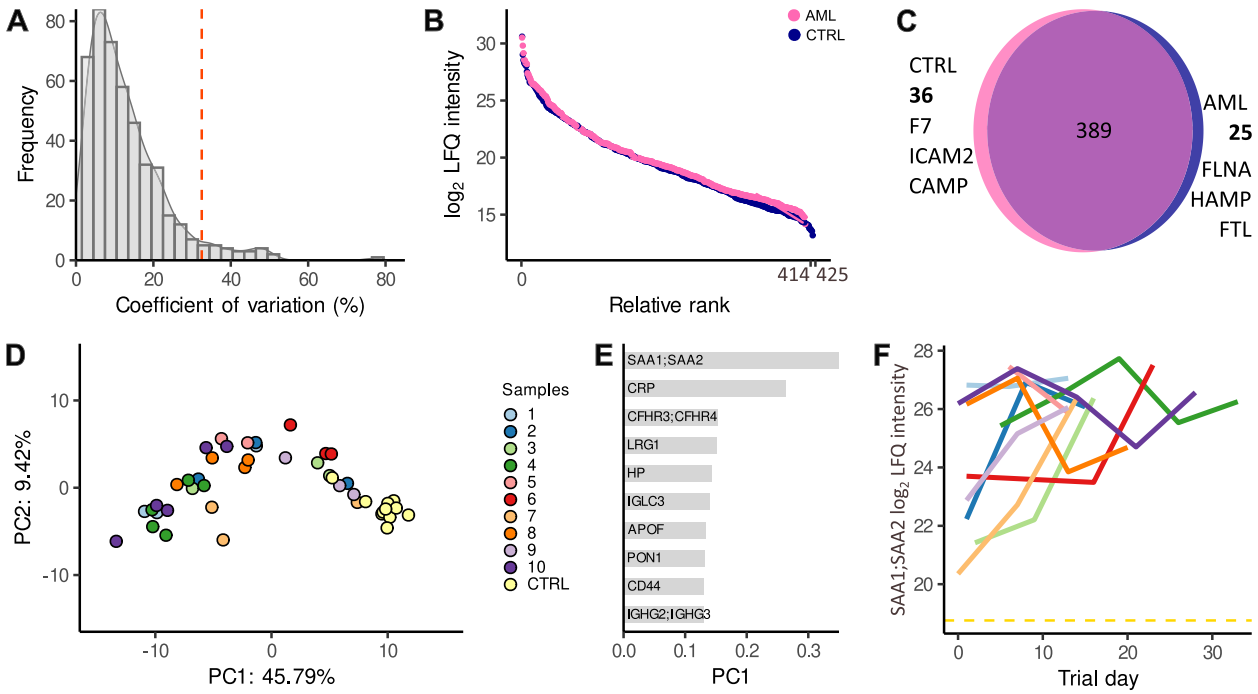

Fig. 2 Global proteomics changes associate with differences in acute phase protein levels. **A** Histogram showing the coefficients of variation (CVs) in proteins detected in the quality control (QC) across the 4 repeated injections in the study used to evaluate analytical variability. **B** Distribution of proteins quantified in both groups, AML (pink) and healthy controls (CTRL, navy) represented as the label free quantification (LFQ) intensities against the ranking of relative intensity of the proteins per group. **C** Venn diagram comparing proteins identified in AML patients or healthy controls (CTRL), 389 shared proteins. Subset of proteins uniquely quantified in the AML or CTRL groups are specified and example proteins are shown per each group. **D** Principal component analysis scores plot based on all 45 samples showing global differences in their plasma proteome. Samples were colored by the individual AML patients (patient 1 to 10) and healthy control group. **E** Top 10 proteins with the highest absolute loading score along principal component 1, highlighting that the differences in the plasma proteome between samples are largely driven by inflammation proteins such as SAA1, CRP and HP. **F** Label free quantification intensities for AML patients over time for peptides shared between serum amyloid A-1 protein and serum amyloid A-2 protein (SAA1;SAA2)

each other and visualized in a correlation map. Exploration of strong positive correlations ($r \geq 0.8$) between proteins revealed two large clusters of proteins, as well as platelet and hemoglobin clusters which we visualized in networks (Supplemental Fig. S3). We explore the platelet and hemoglobin clusters given the known anemia and thrombocytopenia in these patients to confirm expected co-expression patterns between associated proteins. Firstly, we observed a cluster of 4 hemoglobin proteins (hemoglobin subunits alpha 1 and beta (HBA1 and HBB)) (Fig. 4B). Secondly, the platelet cluster consisted of 3 proteins (PF4; PLT factor 4 variant 1 (PF4;PF4V1) and pro-PLT basic protein (PPBP)) (Fig. 4C). As expected, we observed similar longitudinal patterns for all proteins within a cluster for individuals, as their trajectories showed similar declines and peaks throughout the treatment periods (Fig. 4D and Fig. 4E). Additionally, we also found that these protein levels in patients were consistently lower compared to healthy controls.

In addition, we observed two other large clusters, namely cluster 1 ($n = 56$ proteins) and cluster 2 ($n = 44$ proteins). Analysis of proteins in these two clusters showed enrichment for liver tissue-associated proteins (Table S1I). In general, cluster 1 contained protein signatures of acute phase response proteins (CRP, SAA1, HAMP), complement proteins (C9 and C6), coagulation proteins (coagulation factor 10 and 11) as well as cell signatures from monocytes (CD14) and endothelial cells (VCAM1, LYVE1) (Fig. 5A, Table S1J). For all proteins in this network, we observed a general trend of increased protein levels in AML patients compared to the controls (Fig. 5C). Cluster 2 contained proteins involved in serine-type endopeptidase inhibitor activity (Heparin cofactor II, SERPIND1; alpha-1-antichymotrypsin, SERPINA3), regulation of plasma lipoprotein particle levels (Phosphatidylcholine-sterol acyltransferase, LCAT; apolipoprotein A-I, APOA1), and complement proteins, including regulation of complement activation and the alternative pathway (complement component 3 and 5) (Fig. 5B, Table S1K). Overall, the proteins in cluster 2 showed lower levels in AML patients compared to controls (Fig. 5D). Hence, proteins with different dynamics

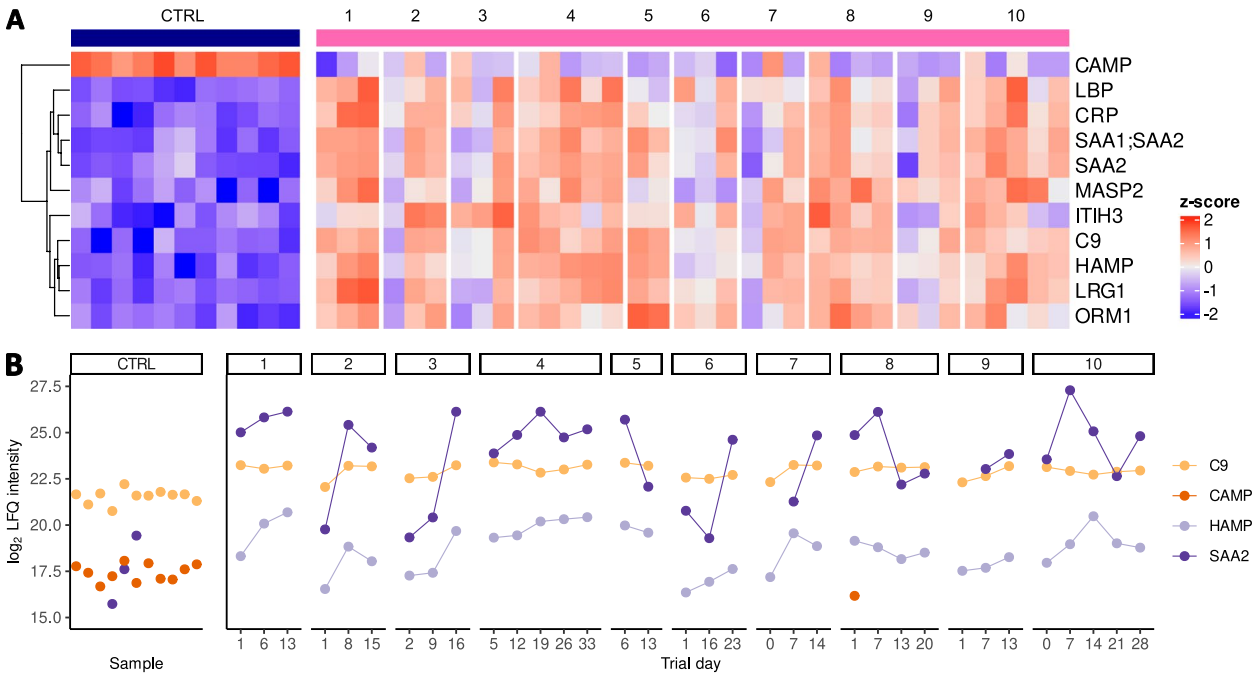

Fig. 3 Acute phase proteins are significantly increased in AML patients during platelet transfusion treatment**. A** Hierarchically clustered heatmap depicting the 11 significantly different proteins on the rows and individual AML patients and healthy controls (CTRL) on the columns. The 11 significantly altered proteins were identified through the comparison of all samples from AML patients to those of healthy controls. Label free quantification intensities are Z-scored. **B** Longitudinal LFQ intensities showing the protein levels of cathelicidin antimicrobial peptide (CAMP), serum amyloid A-2 protein (SAA2), complement component C9 (C9) and hepcidin (HAMP). Non-imputed LFQ intensities are shown for all 10 patients and controls (CTRL)

between AML patients and controls clustered together in networks based on their trajectories highlighting alterations in AML patients compared to a healthy baseline.

Discussion

Chemotherapy-induced bone marrow aplasia in AML patients increases their risk of anemia, bleeding and infections. Hence, constant monitoring of PLT counts and Hb levels is carried out to guide supportive care treatment, PLT and RBCs transfusions, for these patients. Here, we used MS-based plasma profiling in AML patients throughout their transfusion treatment period to evaluate whether circulating plasma proteins could be associated to the overall state of AML patients during this time. In line with previous reports, which described the upregulation of pro-inflammatory cytokines during remission induction chemotherapy in AML patients [39], we observed increased levels of plasma proteins associated with inflammation and the complement system during the patients' treatment period compared to healthy controls. The highest increase was observed in HAMP levels, which has previously been reported in AML patients and is likely driven by a dysregulation in iron metabolism and inflammation [40, 41].

The only protein with significantly lower levels in AML patients compared to controls was CAMP (hCAP-18). This decrease in protein levels in AML patients is likely caused by the bone marrow aplasia experienced during their treatment phase, as neutrophils and bone marrow are the major sources of this protein [42, 43]. Importantly, it has been seen that a decrease in hCAP-18 levels in plasma is associated with poor prognosis in critically ill patients [44, 45]. Therefore, tracing levels of this protein, as previously suggested for children with hematologic malignancies [46], may be used as a guiding parameter, besides neutrophil counts, for myelopoiesis in patients.

The individual-specific protein dynamics observed amongst AML patients prompt us to characterize the protein patterns in the cohort with correlation analysis. This showed distinct clusters of proteins associated with either PLTs or Hb subunits demonstrating the robustness of this approach [47]. Additionally, two large clusters of correlated proteins with either increased or decreased levels of proteins in AML patients compared to controls were found. Amongst these proteins, coagulation proteins F10 and F11 had increased levels, whereas proteins with inhibitory effects on coagulation factors including SEPRIND1 and SERPING1 showed decreased levels in AML patients compared to controls. Moreover, F7

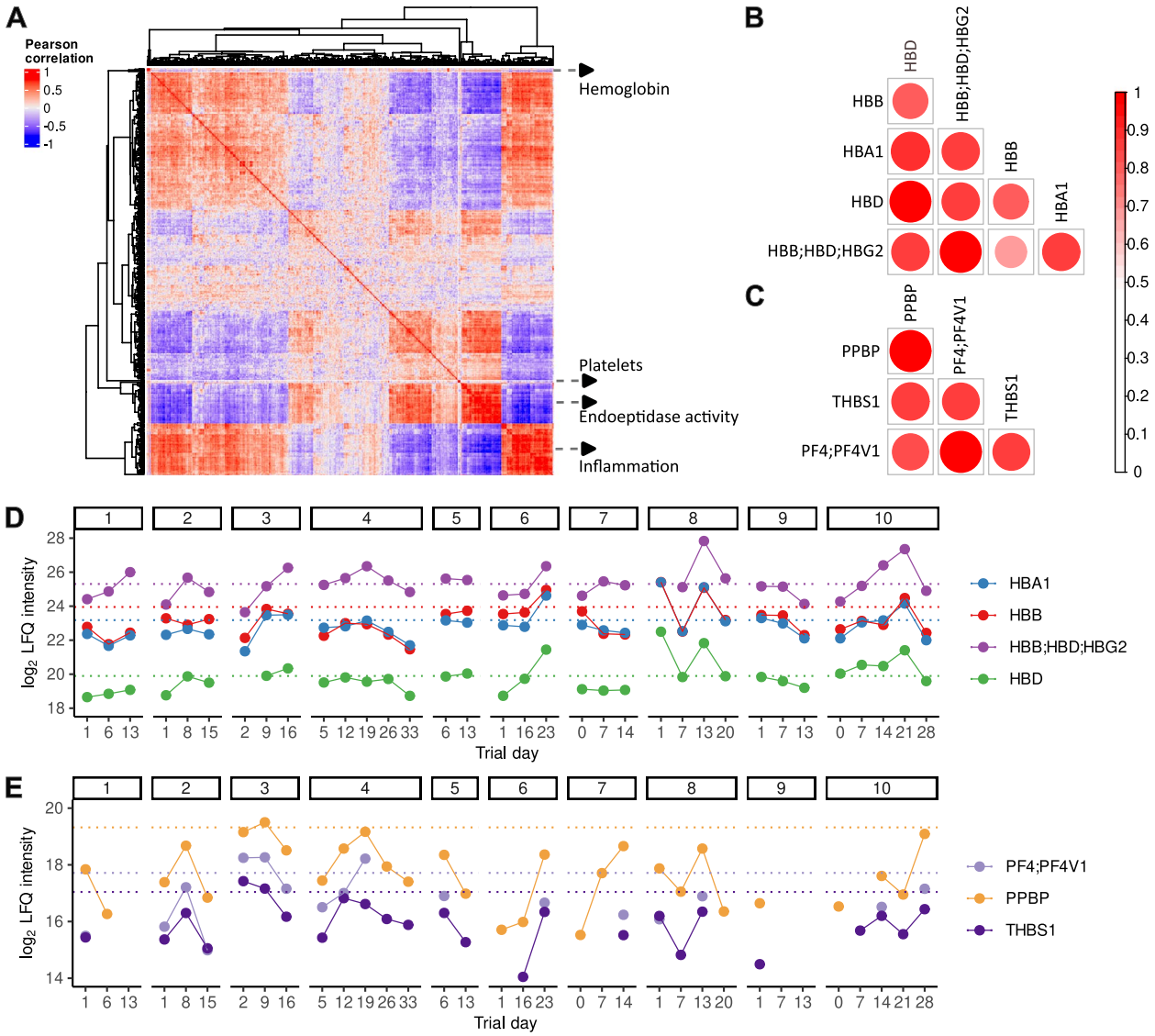

Fig. 4 Protein dynamics describe clusters of proteins with co-expression patterns. **A** Heatmap of Pearson correlations, representing the pairwise correlations between all quantified proteins, determined based on imputed data. Red indicates positive correlations between proteins and blue indicates negative correlations. Highly correlating groups of proteins (> 0.8) were manually annotated. **B** Zoom in correlation plot representing the pairwise correlation of proteins associated with hemoglobin. **C** Zoom in correlation plot representing the pairwise correlation proteins associated with platelets. **D** Longitudinal label free quantification (LFQ) intensities showing the protein levels of highly correlating proteins depicted in B for all individual AML patients. **E** Longitudinal LFQ intensities showing the protein levels of highly correlating proteins depicted in C for all individual AML patients. Mean healthy control levels are indicated with dotted horizontal lines

was only quantified in healthy controls which may indicate decreased levels of F7 in AML patients compared to healthy controls. Because F7 is near the lower limit of the protein depth in healthy controls, it is likely that this protein is also slightly decreased in AML patients, thus falling below the limit of detection in the current study. Similarly, proteins in the complement cascade were observed in these two major clusters. C3, which is specific for the alternative pathway, showed decreased levels, whereas proteins from the terminal pathway (C6, C9, C8A) and classical pathway (C1S, C1R) had increased levels. Taken together, these results point towards a disbalance in the interplay between the coagulation and complement systems. This disarray of proteins from systems characterized by functional crosstalk could contribute to the disconnection between bleeding complications, PLT counts and PLT transfusions in patients. The next step would be to apply this technique in a larger

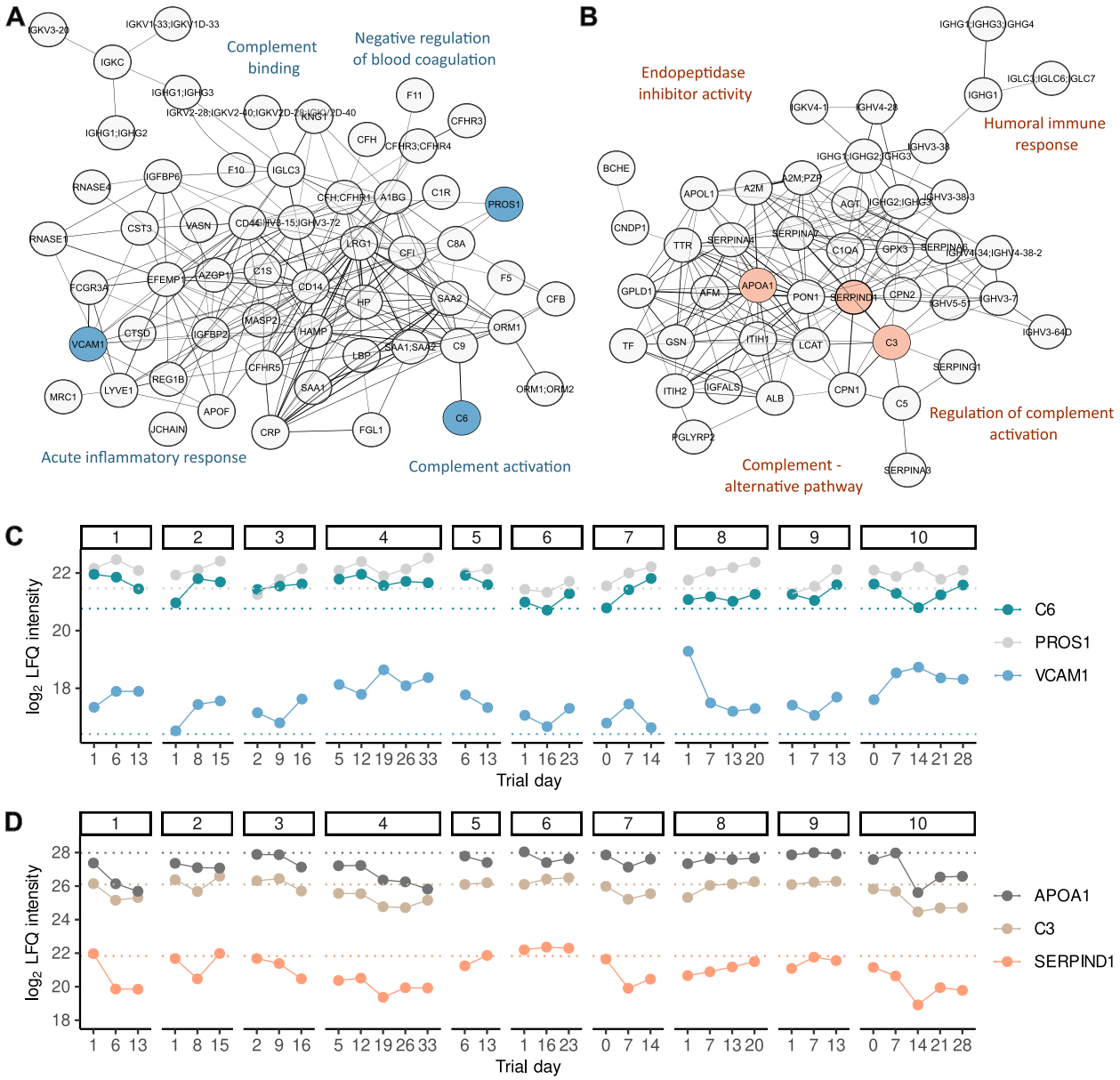

Fig. 5 AML patients have different protein dynamics compared to healthy controls. **A** Network of proteins from main cluster 1 (inflammation), highlighted in Fig. 4A. **B** Network of proteins from main cluster 2 (endopeptidase activity), highlighted in Fig. 4A. Proteins are depicted as nodes and their Pearson correlation coefficients as edges. Functional enriched pathways with example proteins are highlighted. **C** Longitudinal LFQ intensities showing the protein levels of complement component 6 (C6), vitamin K dependent protein S (PROS1) and vascular cell adhesion protein 1 (VCAM1), proteins in A, for all individual AML patients. **D** Longitudinal LFQ intensities showing the protein levels of apolipoprotein A-I (APOA1), complement component 3 (C3) and heparin cofactor 2 (SERPIND1), proteins in B, for all individual AML patients. Mean healthy control levels are indicated with dotted horizontal lines

cohort in order to assess its potential translational impact on the clinical care of these patients.

Notably, these results may also suggest general dysregulation of protein production in the liver as both statistical and correlation analysis revealed a strong signature of liver proteins. This liver dysregulation can be a result of chemotherapy treatment and overall inflammatory state of the patients as abnormally high liver function tests have been reported in AML patients [48]. Moreover, the dysregulation of the coagulation and complement system observed here has been reported in liver disease, including increased levels of acute phase proteins and decreased levels of complement associated proteins [49, 50]. Furthermore, alterations regarding APOA1, PON1

and SAA1 observed here have been previously reported during inflammation [51–53]. Similarly, the alterations of PON1, APOA1 and LCAT presented here are in agreement with decreased expression of lipid metabolism genes reported as chemotherapy effect in hepatocytes [54] and a general dysregulation of proteins involved in lipid metabolism reported in cancer patients undergoing chemotherapy [54, 55]. Importantly, an increase in lipid peroxidation has been observed after transfusion of PLT concentrates [56]. This potential unbalanced lipid metabolism should be further explored as it can have an important short-term effect weakening PLT function [57] and could be linked to a reported increased risk of atherosclerotic cardiovascular disease in cancer survivors treated with chemotherapy [58].

A limitation of this study is the small number of patients included. Nonetheless, analysis of clinical parameters showed agreement with previous literature on transfusion thresholds from larger cohorts [59–68], suggesting this cohort could be representative of the patient population. At the same time, the range of transfusion triggers observed in this cohort is comparable to the wide range of triggers applied in practice for RBCs and PLT transfusions in haemato-oncological patients [69–71]. Here, we purposely selected a population with no previous transplants, transfusions and ongoing infections to limit additional factors that may impact the plasma proteome [72, 73]. This study provides a first step towards future research, which will include a larger cohort with groups of hemato-oncological patients to further categorize sub-groups responses. This could allow for estimation of plasma protein levels associated with transfusion-free survival or bleeding-free survival in the hemato-oncological patient population. Secondly, the samples from AML patients were collected as part of the PREPAReS study (2010–2016) [28, 29] and kept at -80 °C until thawing for this analysis, whereas the healthy controls were collected in 2022. The present study involves samples from patients in different centers with sample collection protocols that may vary compared to healthy controls. We expect limited impact from these variations as previous studies have shown that the effects of sample collection on plasma profiles remain limited to PLT, fibrinogen and RBC signatures [47] and not the inflammatory response, lipoprotein remodeling nor complement proteins reported in this study. Additionally, it has been reported that long term storage differences have minimal effect on plasma protein results analyzed by MS [73], hence the observations reported here and their comparison to controls are reliable.

Conclusion

In summary, we describe longitudinal plasma proteome changes in AML patients receiving supportive care transfusions during and after chemotherapy treatment. We found clustering of individuals, suggesting individual-specific plasma profiles that can be traced during treatment. As previously reported, our results highlight inflammation as a main driver during the transfusion treatment period in these patients. Importantly, we also report changes in proteins associated with endopeptidase activity, plasma lipoprotein remodeling, coagulation and complement system in AML patients during their transfusion treatment phase. Further research in a larger cohort is needed to evaluate these potentially affected biological pathways and their link to transfusion effectiveness and bleeding complications in these patients.

Abbreviations

AML	Acute myeloid leukemia
LFQ	Label-free quantification
MS	Mass spectrometry
CV	Coefficient of variation
PLT	Platelet
Hb	Hemoglobin
PREPAReS	Prospective analysis of patients from the Pathogen Reduction Evaluation & Predictive Analytical Rating Score
RBC	Red blood cell
RT	Room temperature
PCA	Principal component analysis

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s41231-024-00189-5.

Supplementary Material 1.

Supplementary Material 2.

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Authors' contributions

ERS and DMS made substantial contributions to conception and design, data analysis, interpretation and visualization of data, drafted and revised the article. ERS made substantial contributions to the methodology. ICK, AJH made substantial contributions to the data analysis and critically reviewed the manuscript. ERS and CvdZ performed experiments and acquisition of data. JV, PvdM, PY, JLHK and MvdB made substantial contributions to conception and design, interpretation of the data, and revised the manuscript critically. All authors approved the final version to be published.

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Availability of data and materials

Anonymized processed data is available in the Supplemental Table 1B and scripts used for the proteomic analysis and visualization are available on GitHub at https://github.com/evasmit/PlasmaProteomicsPreparesPilot.

Declarations

Ethics approval and consent to participate

All participants gave written informed consent before the randomization procedure or any other study-related procedure. The protocol and methods used was published previously and the study was registered at the Netherlands National Trial Registry under number NTR2106 as well as at clinicaltrials.gov under number NCT02783313. Briefly, the protocol was approved centrally and by site institutional review boards and according to the criteria outlined in the Declaration of Helsinki and Good Clinical Practice guidelines.

Consent for publication

Informed consent was obtained from patient(s).

Competing interests

None of the authors have competing interests in relation to this publication.

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