Statistical analysis plan:

Official Title:
“The Effect of Plasma Donation Frequency on Plasma Protein Composition, Inflammation Markers and Psychological Distress - a Randomized Controlled Trial”

Study title:
“The effect of plasma donation frequency on plasma proteins and donor safety: a randomized controlled trial”

NCT05179200

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Introduction

Background and rationale

The demand for plasma-derived medicinal products (PDMPs) is increasing and plasma collection must be expanded globally to meet this demand (1). In the US, plasma donors can donate up to 104 times per year with minimum 48-hour donation intervals (2). In Europe, up to 33 plasma donations annually and a minimum 96-hour donation interval is recommended (3) which was replaced by 1-week donation intervals in the most recent edition (4).

A scoping review of the effect of plasma donation frequency on donor health concludes that there is a lack of evidence to establish a safe upper limit for plasma donation frequency. More experimental studies are therefore needed (5).

The first RCT on donation frequency and donor health found a large and clinically relevant reduction in ferritin and IgG concentrations in plasma donors donating plasma twice per week of 60 and 38 %, respectively (6).

In this paper, our aim is to investigate the effect of different plasma donation frequencies; high-frequency plasma donors (HFDPs) who donate plasma 3 times per 2 weeks, and regular-frequency plasma donors (RFPDs), who donate plasma 1 time per 2 weeks, on plasma proteins, haemoglobin, ferritin, adverse events and self-reported psychological symptoms compared to controls donating whole blood every 3 months. Additionally, the recovery of these biomarkers after the donations will be investigated.

Research question: What is the effect on the concentrations of plasma proteins, ferritin, and haemoglobin, in donors donating 650 ml plasma (excl. AC) 3 times per 2 weeks compared to donors donating plasma once per 2 weeks and controls donating whole blood?

Objectives

Research hypothesis: High-frequency plasma donation of 650 mL of plasma 3 times per 2 weeks is non-inferior to both plasma donation of 650 mL of plasma 1 time per 2 weeks and whole blood donation in terms of donor health based on differences in the concentrations of TSP, IgG, and various other specific plasma proteins.

Primary objective:
(1) To compare the concentrations of TSP (g/L) and IgG (g/L) at baseline, during a 16-week donation period, and after a 4-week follow-up period between the HFPDs, RFPDs, and controls.

Secondary objectives:
(1) To compare the concentrations of other plasma proteins, including IgG subclasses, between the HFPDs, RFPDs, and controls.
(2) To compare symptoms of psychological distress before and after the donation period between the HFPDs, RFPDs, and controls using the Hopkins Symptoms Checklist 25 (HSCL-25).
(4) To compare the dropout rate and reasons for dropout between the HFPDs, RFPDs, and controls.

(5) To compare the AEs, and evaluate their relationship to plasma/blood donation, between HFPDs, RFPDs, and controls.

Study methods

Trial design

A randomized controlled study, parallel-group, where 120 participants will be randomized 1:1:1 to donate 650 mL plasma excluding AC either three times per 2 weeks, “High-frequency plasma donors” (HFPDs) or once per 2 weeks “Regular-frequency plasma donors” (RFPDs), or to the control group donating whole blood every 3 months, for 16 weeks.

Trial population

Established male blood- and plasma donors aged between 18 and 64 years will be screened for eligibility. Donors must have a history of at least one previous plasma donation and meet the eligibility criteria for both whole blood and plasma donation by plasmapheresis including sufficient levels of Hb, TSP, and IgG. The estimated blood volume (EBV) determined by the ISCH formula (7) must be at least 4500 mL. Donors with a history of repeated measurements (>2) of haematocrit >50 % before enrolment will be excluded.

All participants will provide written informed consent. This study will be conducted from January 2022 to June 2024 at four different donation sites (Elverum, Hamar, Lillehammer and Gjøvik) at the Blood Centre, Innlandet Hospital Trust, Norway. The study is approved by the Regional Committee for Medical and Health Research Ethics Southeast Norway (2021/238929/REC Southeast A) and performed according to the Declaration of Helsinki. The study is registered at clinicaltrials.gov (identifier NT NCT05179200).

Intervention

The plasmapheresis procedure will be performed using the Aurora Plasmapheresis machine, Fresenius Kabi. The plasma donation volume is set at 720 mL, including AC, which corresponds to approximately 650 mL plasma, excluding AC, assuming a haematocrit of 44% and an ACR of 100:6.

Data collection

Blood samples and analyses

Blood samples will be collected from each participant immediately before the planned plasma- or blood donation in total 10 times; every 2 weeks from baseline during the donation period and 2 and 4 weeks after the donations.

Data on psychological distress

Data on psychological distress in donors will be collected by Hopkins Symptoms Checklist 25 (HSCL-25), at week 0 (W0) and week 17 (W17). It consists of 25 questions that will be rated on a 4-point scale from “Not at all (1)” to “Extremely (4)”) (8) to how the donor felt the preceding 2 weeks. A mean score will be calculated based on all 25 answers and a cut-off of 1.75 will be used to define psychological distress.

Data on adverse events

Adverse events will be categorized according to “Standards for Surveillance of Complications related to blood donations (9). Adverse events will be reported as a number per procedure and per donor.

Variables

Outcomes measures

Change from baseline at week 1 (W1) until the endpoint of the last donation at week 1/17 (W16/17) of the following biomarkers:

- **Plasma proteins:** Total serum protein, g/L, IgG, g/L, IgG subclasses 1-4, g/L, IgA, g/L, IgM, g/L, albumin, g/L, transferrin, g/L, CRP, mg/L, lipoprotein A, mg/L and Apo lipoprotein B, g/L.
- **Other biomarkers:** Ferritin, µg/L, haematocrit, %, and haemoglobin, g/dL.

The number of participants temporarily deferred due to low concentrations of immunoglobulin G (<6.0 g/L) and/or total serum protein (<60.0 g/L).

The number of dropouts and reasons for dropout.

The difference from W0 to W17 in the mean overall score and score for each item of the HSCL-25, including the proportion of participants with an overall score ≥1.75.

The frequency of adverse events.

Independent variables

Exposure variables:

- Study group identity, three levels.

Statistical analysis

Stata (Stat Corp, College Station, TX, USA) statistical software package will be used for the statistical analyses.

Outcome variables will be checked for normality and normally distributed data will be expressed as mean (SD) and non-normally distributed data as median (min-max or IQR). 95% confidence intervals will be used.

The mean difference of concentrations of the biomarkers will be calculated by study group between W1 and W16, and the recovery of the biomarkers in W18 and W20 will be described.

We will use global ANOVA analyses or generalized linear mixed effects models with time and group as independent variables with interaction terms between them. Dependent variables will be the concentrations of the different biomarkers and the random variable will be participant ID. Test of normality of the residuals will be performed using QQ-plot. Trajectories of the biomarker concentrations between the different groups will be described.

The total number of adverse events will be calculated and the time until AEs and/or dropouts between the groups will be compared using Cox proportional hazards models.

The difference in total HSCL-25 score between W0 and W17 will be compared by linear regression and by comparing proportions scoring above the cut-off value of 1.75 using binomial regression models. The difference in score per single HSCL-25 question will also be examined.

Data will be analyzed both with intention-to-treat and per-protocol analyses, to reduce potential bias from the healthy donor effect (10, 11). The study participants who complete at least 87.5% of the scheduled donations in the intervention groups will be included in the per-protocol approach.

Results

Suggested tables, figures, and supplementary:

Figures

Figure 1: Flow chart of the participants by study group through each stage of the trial:

- Assessment for eligibility.
    - Exclusion with reasons.
- Participants who were randomly assigned.
    - Dropouts with reasons or lost to follow-up.
- Participants who completed intervention as allocated (≥87.5%): per protocol group.
- Participants who did not complete intervention as allocated: intention to treat group.
    - Reasons for not completing the intervention.
- Participants who were included in the main analysis (intention-to-treat group) or per-protocol analysis.

Figure 2: Fitted lines or margins plot (following mixed effects models) of concentrations of primary and key secondary outcomes every 2 weeks during the donation period from W1 to W16 with CI.

Figure 3: Fitted lines or margins plot (following mixed effects models) of the recovery of concentrations of primary and key secondary outcomes from W16 to W18 and W20.

Figure 4: Survival analysis of time until adverse events, low concentrations of primary outcomes, and/or dropout by study group.

Figure 5: Concentration of all protein biomarker outcomes as a proportion of the total protein by study group and time.

Tables

Table 1: Baseline characteristics

	HFPDs (n=)	RFPDs (n=)	Controls (n=)
Age (years)			
Height (cm)			
Weight (kg)			
BMI (kg/m^2)			
Estimated blood volume (mL)			

Blood type • A • O • B • AB			
Donation history • Whole blood • Plasma • Platelets			
Education • Less than 10 years of primary school • Primary school • Secondary school • University or college (up to 4 years) • University or college (more than 4 years) • Other			
Marital status • Single • Married • Cohabitant • Separated • Divorced • Other			
Main activity • Work (full time) • Work (part-time) • Stay at home • Sick leave • Leave • Disability insurance • Student • Unemployed • Retired • Other Occupation			
Plasma proteins • TSP (g/L) • IgG (g/L) • IgG1 • IgG2 • IgG3 • IgG4 • …			
Ferritin (µmol/L)			
Haemoglobin (g/dL)			

Data are means (SD), n (%), or median (min-max or IQR). Parts of Table 1 as supplementary.

Table 2: Concentrations of primary and key secondary outcomes and difference from W1 to W16 and to the recovery period (W18 and W20) within study group and compared to controls.

Time point	Outcome	HFPDs (n=)	Difference from	RFPDs (n=)	Difference from	Controls (n=)	

		Mean (SD)	Difference from W1 (95 % CI)	controls (95% CI)	Mean (SD)	Difference from W1 (95 % CI)	controls (95% CI)	Mean (SD)	Difference from W1 (95 % CI)	p-value
W16	TSP, g/L									[1, 2, 3]
	IgG, g/L									
	IgG1, g/L									
	IgG2, g/L									
	IgG3, g/L									
	IgG4, g/L									
W18 (recovery)										
W20 (recovery)										

Data are means (SD). Extended table in supplementary for other outcomes. [1]p<0.05 between HFPDs and RFPDs, [2]p<0.05 between HFPDs and controls, [3]p<0.05 between RFPDs and controls.

Table 3: Measurements of low concentrations of primary outcomes by study group.

	Week	1	3	5	7	9	11	13	16	18 (recovery)	20 (recovery)
IgG<6	HFPDs RFPDs Controls										
TSP<60	HFPDs RFPDs Controls										

Table 4: Number of participants temporarily deferred from the intervention due to low concentrations of primary outcomes.

Deferral (duration)	HFPDs (n=)	RFPDs (n=)	Controls (n=)
No deferral			
1 (2 weeks)			
2 (4 weeks)			
3 (6 weeks)			
4 (8 weeks)			

Table 5: Preferred donation frequency after the donation period.

	HFPDs (n=)	RFPDs (n=)	Controls (n=)
Several times per week			
Weekly			
Biweekly			
Monthly			
Every 1-3 months			
Less often than every 3 months			
Don't want to donate plasma anymore			

Data are n (%)

Table 6: Psychological distress before and after the donation period by study group:

	HFPDs (n=)			Effect	RFPDs (n=)			Effect	Controls (n=)	
	W0	W17	Diff		W0	W17	Diff		W0	W17
HSCL-1										
HSCL-…										
Total score of HSCL-25										
HSCL ≥1.75										

Data are mean (95% CI) and % (95% CI). p-values for trend differences.

Supplementary

Supplementary Table 1: Concentrations of other outcomes and difference from W1 to W16 and to the recovery period (W18 and W20) within study groups and compared to controls.

Time point	Outcome	HFPDs (n=)		Difference from controls (95% CI)	RFPDs (n=)		Difference from controls (95% CI)	Controls (n=)		p-value
		Mean (SD)	Difference from W1 (95 % CI)		Mean (SD)	Difference from W1 (95 % CI)		Mean (SD)	Difference from W1 (95 % CI)	
W16	…									[1, 2, 3]
W18										
W20										

[1]p<0.05 between HFPDs and RFPDs, [2]p<0.05 between HFPDs and controls, [3]p<0.05 between RFPDs and controls.

Supplementary Table 2: P-values of the main effects from global ANOVA analyses or generalized linear mixed effects models - interaction between time and concentrations of primary outcomes and key secondary outcomes.

	Group	Time	Group x Time	HFPDs vs Controls	RFPDs vs Controls	HFPDs vs RFPDs
TSP						
IgG						
…						

p-values from mixed effects models.

Supplementary Table 3: Number of adverse events by category and study group.

Adverse events	HFPDs (n=, n donations)	RFPDs (n=, n donations)	Controls (n=, n donations)

A. Local symptoms 1. Blood outside vessel • Haematoma • Arterial puncture 2. Arm pain: • Nerve injury/irritation 3. Localized infection/inflammation of vein or soft tissues 4. Other major vessel injury • Deep Venous Thrombosis (DVT) • Arteriovenous fistula • Compartment syndrome • Brachial artery pseudoaneurysm			
B. Generalized symptoms – Vasovagal Reaction • Vasovagal reactions, no loss of consciousness (LOC) • Vasovagal reaction, loss of consciousness			
C. Related to apheresis • Citrate reactions • Haemolysis • Air embolism			
D. Allergic reactions • Local allergic reaction • Generalized (anaphylactic) reaction			
E. Other serious adverse events • Acute cardiac symptoms (other than myocardial infarction or cardiac arrest • Myocardial infarction • Cardiac arrest • Transient ischemic attack (TIA) • Cerebrovascular accident			
F. Other • Anemia			
Total			

Number of adverse events (grade 1-5). The adverse event rate per participant and per 50 procedures by study group will be calculated and described in the results.

Supplementary Table 4: Description of trial deviations.

Ethical considerations and data storage

This study will adhere to the Declaration of Helsinki and has been approved by the Regional Committee for Medical and Health Research Ethics Southeast Norway (2021/238929/REC Southeast A). All participants provided written informed consent and participation could be withdrawn at any time point. All data and analyses are securely stored and managed on a safe dedicated server at Innlandet Hospital Trust. This study received funding from Innlandet Hospital Trust, Norway.

Timelines and Milestones

	Jun 24	*July 24*	*Aug 24*	*Sep 24*	*Oct 24*	*Nov 24*	*Dec 24*
Statistical analysis plan	x						

Complete dataset			x					
Introduction	x		x					
Methods				x	x			
Statistics and analyses			x	x	x	x		
Results					x	x		
Discussion						x	x	
Submission								x

Authorship

Authorship for this paper will be determined based on the Vancouver Convention on authorship (12):

Morten Haugen (MH, corresponding and first author)

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Responsibilities

Completion of data set	MH, TAS
Drafting introduction and methods section	MH, LSHNM
Statistics and analyses	MH, TAS
Presentation of results	MH
Drafting results section	MH, TAS
Drafting discussion section	MH, KM, LSHNM, TAS
Evaluation of paper draft	MH, KM, LSHNM, TAS
Authoring paper and final submission	MH

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