Statistical Analysis Plan (SOP)

Parent study Title: "The Effect of Plasma Donation Frequency on Plasma Protein Composition, Inflammation Markers and Psychological Distress - a Randomized Controlled Trial"

Sub Study Title: "The Effect of Plasma Donation Frequency on PFAS Concentrations – a randomized controlled trial"

ClinicalTrials.gov Reference: NCT05179200

Date: 01.09.2025

Introduction

Background and Rationale

Poly- and perfluoroalkyl substances (PFAS) are synthetic compounds used for their water- and fat-repellent properties in products such as textiles, food packaging, cosmetics, and electronics [1-3]. PFAS are highly persistent in the environment and the human body, with long biological half-lives of 1-5 years [4]. As a result, they are widely detected in drinking water, soil, and food. Exposure in humans primarily occurs through the ingestion of contaminated water and food, as well as through everyday consumer products such as non-stick cookware, water-repellent clothing, food packaging, dental floss, and cosmetics. PFAS are absorbed into the bloodstream and bind to proteins rather than fat [3, 5-7], leading to bioaccumulation over time [1, 8]. This accumulation has been linked to adverse health effects, including immune suppression, hormonal disruption, elevated cholesterol, and potential carcinogenicity [9].

PFAS exposure is primarily assessed through blood concentrations. A recent Swedish study investigated PFAS exposure in the general population and analysed samples from 60 blood donors [10]. They detected 26 of 30 different PFAS and observed PFHxS-lin, PFOS (linear and branched), PFOA-lin, PFNA, and PFDA in all samples, and found the highest concentrations of PFOS-lin, PFOS-br, PFOA-lin, and PFHxS-lin.

A previous RCT in Australian firefighters, with high exposure to PFAS through firefighting foams, demonstrated reductions in PFAS concentrations following plasma donations every 6 weeks and blood donations every 12 weeks [11].

In this study, we aim to investigate the effect of different plasma donation frequencies on PFAS concentrations in blood donors.

Objectives

To compare the plasma concentration of different PFAS at baseline and after 16 weeks of donations between high-frequency plasma donors (HFPDs), donating plasma 3 times every 2 weeks, regular-frequency plasma donors (RFPDs), donating plasma once every 2 weeks, and a control group (controls) donating whole blood every 3 months.

Study Methods

Trial Design

This is a randomized controlled trial, parallel-group, where 120 male blood donors were randomized 1:1:1 for donation over 16 weeks to:

- High-frequency plasma donors (HFPDs) donating plasma 3 times every 2 weeks (in total 24 times)
- Regular-frequency plasma donors (RFPDs) donating plasma once every 2 weeks (in total 8 times)
- The control group (controls) donating whole blood every 3 months (in total 2 times)

The study was conducted at the Blood Centre of Innlandet Hospital Trust from January 2022 to July 2024.

Trial Population

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

All participants provided written informed consent. The study was approved by the Regional Committee for Medical and Health Research Ethics in Southeast Norway (2021/238929/REC Southeast A) and was conducted in accordance with the Declaration of Helsinki. The study is registered at clinicaltrials.gov (identifier: NCT05179200).

Intervention

The plasmapheresis procedure was performed using the Aurora Plasmapheresis machine, Fresenius Kabi. The plasma donation volume was 720 mL, including AC, which corresponds to approximately 650 mL plasma, excluding AC, assuming a haematocrit of 44% and an ACR of 100:6. The whole blood donations were 450 mL of whole blood.

Data Collection

Baseline data were collected through questionnaires and from the blood centre's database, LabCraft.

Blood Samples and Analyses

One tube of 6 mL EDTA plasma was collected non-fasting per participant immediately before the plasmapheresis procedure, at:

- Baseline week 1 (W1): Before the first donation in the study, after at least two months of no donation.
- End point week 18 (W18): Two weeks after the final donation.

The plasma samples were centrifuged at 2200×*g* for 10 min immediately after collection, transferred into aliquots of 500 µL, and frozen at −40°C within 3 hours after collection. Within 3 months of storage, the samples were transferred to storage at −80°C until analysis. The samples were shipped on dry ice to the laboratory for analysis. PFAS analyses were conducted at the Environmental Pollutant Laboratory, University Hospital of North Norway, Tromsø, Norway, according to the method described by Huber and Brox [12]. Samples were analysed in two batches of 96 samples in 2023 and 122 samples in 2024.

Variables

Outcome Measures

Difference in plasma concentrations between W1 and W18 of the following PFAS:

Primary outcomes:

- Perfluorooctanoic acid (PFOA)
- Perfluorooctane sulfonic acid (PFOS)*
- Perfluorohexane sulfonic acid (PFHxS)*
- Perfluorononanoic acid (PFNA)
- Sum of PFOA, PFOS, PFHxS and PFNA [13]

Secondary outcomes:

- Perfluorobutanoic acid (PFBA)
- Perfluoropentanoic acid (PFPeA)
- Perfluorohexanoic acid (PFHxA)
- Perfluoroheptanoic acid (PFHpA)
- Perfluorodecanoic acid (PFDA)
- Perfluoroundecanoic acid (PFUDA)
- Perfluorododecanoic acid (PFDoDA)
- Perfluorotridecanoic Acid (PFTrDA)
- Perfluorotetradecanoic acid (PFTeDA)
- Perfluorobutane sulfonic acid (PFBS)
- Perfluoropentane sulfonic acid (PFPS)
- Perfluorooctane sulfonic acid (PFHpS)*
- Perfluorononane sulfonic acid (PFNS)*
- Perfluorodecane sulfonic acid (PFDS)*
- Perfluorodecane sulfonic acid (PFDoDS)
- Perfluorooctane sulfonamide (PFOSA)*
- 4:2 Fluorotelomer sulfonic acid (4:2 FTSA)
- 6:2 Fluorotelomer sulfonic acid (6:2 FTSA)
- 8:2 Fluorotelomer sulfonic acid (8:2 FTSA)
- 10:2 Fluorotelomer sulfonic acid (10:2 FTSA)

*Analysed as linear and branched isomers, and the sum of these.

Other variables:

- Plasma albumin concentration (as a mediator)

Independent Variables

- Study group identity (HFPDs, RFPDs, control group)

Statistical Analysis

Stata (StataCorp, College Station, TX, USA) will be used for the statistical analyses.

Outcome variables will be checked for normality. Normally distributed data will be expressed as mean (SD), and non-normally distributed data will be expressed as median (min-max and/or IQR). 95% confidence intervals will be used, and a two-tailed p-value < 0.05 will be considered statistically significant.

Samples below the detection and quantification limits:

- The number, n (%), of samples below the limit of quantification (LOQ), as defined per the laboratory protocol, will be calculated.
- The number, n (%), of samples below the limit of detection (LOD) will be calculated.
- Only PFAS (or summarized variables) with a detection rate >70% at baseline will be included in the statistical analysis [14, 15].

For values below the LOD, a common imputation approach will be used where concentrations are replaced by LOD/2 (i.e., one-half the detection limit). Sensitivity analyses may be conducted to evaluate the choice of imputation method for values below the LOD (e.g., LOD/2, LOD/$\sqrt{2}$, or exclusion of values below the LOD) to assess the robustness of the imputation strategies.

Branched isomers of PFHxS, PFHpS, PFOS, PFNS, PFDS, and PFOSA will be calculated as: Branched = sum – linear.

The difference in PFAS concentrations within each group from baseline at W1 to the end point at W18 between the study groups will be estimated using generalized linear models (or similar methods). For continuous outcomes, we will use an identity link function and assume a Gaussian distribution family.

We will also consider analyses using other distribution families based on the skewness of the data, as well as whether to categorize (e.g., dichotomize) the outcome variable.

We will also consider quantile regression and express the effects as the median differences between the study groups.

Bootstrap confidence intervals (95%) for median concentrations and median changes will be calculated using 1,000 resampling iterations to provide robust estimates that are not sensitive to non-normality.

We will also use mediation analyses to estimate the extent to which the effect is mediated through changes in albumin concentration.

Results

Figures

Flow chart of participants and samples, including ITT and PP populations and the number of donations.

Plots showing the concentration of different PFAS from week 1 to week 18.

Tables

Table 1: Baseline characteristics

	HFPDs	RFPDs	Control group
Age			
Height			
Weight			
BMI			
Donation history • Plasma • Whole blood			
Occupation			

Table 2: Baseline concentration of PFAS (incl. branched and linear isomers)

	HFPDs	**RFPDs**	**Control group**
PFBA (ng/mL)			
…			

Table 3: Concentrations of PFAS at W18 and change from W1

PFAS	HFPDs		RFPDs		Control group	
	W18	W18-W1	W18	W18-W1	W18	W18-W1
PFBA (ng/mL)						
…						

*p-value compared to controls at W18

Ethical considerations and data storage

All data and analyses are securely stored and managed on a safe dedicated server at Innlandet Hospital Trust. This study was funded by Innlandet Hospital Trust, Norway.

Supplementary

Table S1: Individual limits of detection (LODi) of PFAS:

PFAS	Range	Median	Mean
PFBA (ng/mL)			
…			

Table S2: Detection frequencies of analysed PFAS in all samples and per intervention group.

PFASs	All samples	All samples W1	All samples W18	HFPD		RFPD		Control	
				W1	W18	W1	W18	W1	W18
PFBA (ng/mL)									
…									

Data are n (%)>LOD (n (%)>LOQ).

Authorship

Authorship for this paper will be determined based on the Vancouver Convention on authorship [16]:

Morten Haugen (MH, corresponding and first author)

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The order of authorship may be revised according to the authors' respective contributions.

Timeline and Responsibilities

Task	**Deadline**	**Responsibility**
Completion and approval of SAP	August 2025	MH, SH, KM, TAS, LSHNM
Completion of data set	August 2025	MH, TAS
Drafting introduction and methods section	August 2025	MH, SH

Task	Deadline	Responsibility
Statistical analysis	September 2025	MH, TAS, LSHNM
Drafting results section	September/October 2025	MH, SH
Drafting discussion section	October 2025	MH, SH
Evaluation of paper draft	November/December 2025	MH, SH, KM, TAS, LSHNM,
Final manuscript submission	December 2025/January 2026	MH

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