*Original article*

# The effect of donor's characteristics on plasmapheresis products: insights for a personalised approach

Lucia Merolle[1], Chiara Marraccini[1], Filippo Vurro[2], Maura Parisi[1], Erminia Di Bartolomeo[1], Laura Bonvicini[3], Serena Broccoli[3], Paolo Giorgi Rossi[3], Roberto Baricchi[1], Thelma A. Pertinhez[1,4]

[1]*Transfusion Medicine Unit, Azienda USL-IRCCS di Reggio Emilia, Reggio Emilia;*
[2]*"Istituto dei Materiali per Elettronica e il Magnetismo" (CNR-IMEM), Parma;*
[3]*Epidemiology Unit, Azienda USL-IRCCS di Reggio Emilia, Reggio Emilia;*
[4]*Department of Medicine and Surgery, University of Parma, Parma, Italy*

**Background** - Anticoagulant concentration in plasma units is extremely variable. Understanding the underlying causes of this variability could help personalise plasmapheresis procedures in order to optimise the risk-benefit ratio. We studied the association between anticoagulant solution A (usually ACD-A, Citrate Dextrose Solution A) volume in plasma units and donor characteristics to build a model to determine the needed weight of the final plasma unit to have an 80% probability of reaching 600 mL net plasma.

**Materials and methods** - We experimentally measured ACD-A in 296 plasma units from an Italian blood donor centre, where machines are set for the collection of 700 g of plasma. Next, we built a statistical model to predict how the final volume of the unit should be set to obtain 50%, 80% or 90% probability of having at least 600 mL net plasma.

**Results** - ACD-A volume was associated with haemoglobin, total proteins and triglycerides. Donors with low haemoglobin reach an 80% probability of at least 600 mL net plasma with units of approximately 690 g, while 720 g are needed for donors with high haemoglobin levels. For total proteins and triglycerides, plasma units may vary within a range of ±20 g.

**Discussion** - Our model, based on easily measurable individual characteristics, makes it possible to customise plasmapheresis procedures by determining the blood volume to be processed for each donor. Tailored plasma donations might result in both a reduction in adverse events and an increase in the quality of collected plasma.

**Keywords:** *plasmapheresis, quantile regression, ACD-A anticoagulant.*

## INTRODUCTION

Plasmapheresis is considered a safe procedure, with low occurrence of adverse effects (AEs)[1,2]. However, it requires processing a large volume of blood and a variable amount of citrate-containing anticoagulant (usually ACD-A, Citrate Dextrose Solution A), which is distributed between the donor and the collected plasma.

New plasmapheresis donation standards have recently been introduced in the European Union and adopted by the member states, including Italy, where at least 600 mL of plasma net from the anticoagulant should be collected in each unit[3,4]. The new Italian standards

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**Correspondence:** Chiara Marraccini
e-mail: chiara.marraccini@ausl.re.it



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involve an increase in the usual volume of plasma collected, and, consequently, in the volume of processed blood.

Recently, we demonstrated that manufacturers' algorithms estimating the net amount of plasma and anticoagulant in plasmapheresis-derived units (based on given parameters, such as ACD-A used during the procedure, haematocrit and volume of processed blood) are rather inaccurate[5]. Furthermore, we showed that the final ACD-A volume in each plasma unit and the amount of ACD-A re-infused to donors are extremely variable. Understanding the causes that influence this variability could help personalise each plasmapheresis procedure in order to minimise the processed blood volume and the citrate re-infused to the donor.

Based on these considerations, we evaluated whether there was any association between ACD-A volume in plasma unit and donor and procedural characteristics in 296 units collected at our Blood Donor Centre. We then built a model based on individual donor characteristics that could be routinely assessed immediately before donation to determine the required weight of the final plasma unit in order to have an acceptable probability of having 600 mL plasma net of the anticoagulant.

## MATERIALS AND METHODS

### Setting

This study is part of a larger project financially supported by the Centro Regionale Sangue Emilia Romagna (CRS, Ospedale Maggiore, Bologna, Italy) and aimed to investigate different aspects of donor and patient safety related to the plasmapheresis procedure. The study was approved by the Ethics Committee of Reggio Emilia, Italy, on 30th November 2016; protocol n. 2016/0028541.

The study was conducted in the main donor centre of the Reggio Emilia Local Health Authority. Plasma collection was performed with Plasma Collection System (PCS2) according to the manufacturer's instructions (Haemonetics Corp., Rosemont, IL, USA). Machines were set for the collection of 700 g of plasma.

Donor selection for plasmapheresis follows strict criteria to reduce the risk of AEs to the donor, in accordance with the Italian Ministerial Decree of 2nd November 2015[4] and the European Directorate on Quality of Medicines (EDQM)[3].

### Study population

Recruitment lasted from February to September 2017. All donors undergoing both annual check-up and plasmapheresis procedure on the same day were considered eligible for the study and asked to participate, after informed consent. Informed consent was obtained from 305 plasma donors, according to the Declaration of Helsinki.

### Sample collection and experimental measurements of ACD-A

Information concerning plasma collection procedures, as well as quantification of ACD-A in the plasma units (derived from citrate concentration using proton nuclear magnetic resonance, $^1$H-NMR), are reported in Vurro *et al.*[5].

Donors were rehydrated with 500 mL of saline solution at the end of the procedure. In 116 clinically motivated cases, the physician decided to administer 250 mL of the saline solution during the procedure and an additional 250 mL at the end.

No unit was wasted for this study, as plasma samples for further analyses were collected without affecting their safety and integrity[5].

We collected 305 plasma units; 9 samples, however, could not be properly quantified with $^1$H-NMR. Experimental assessment of ACD-A, therefore, was obtained for 296 plasma units.

### Main outcomes and variables of interest

Our first aim was to evaluate the main donor characteristics and plasmapheresis procedures that correlate with the measured ACD-A content within the final unit.

We focused on two features of the plasmapheresis process and products that are relevant for the health and wellbeing of the plasma donor and of the patient:

1. ACD-A volume in the unit;
2. net plasma volume in the unit.

These parameters were estimated using an experimental measurement of ACD-A by $^1$H-NMR combining the following variables: total ACD-A used during the procedure, final plasma unit density and weight (**Table I**).

Our second aim was to build a model to predict the probability of having at least 600 mL of net plasma in the unit. We considered as independent variables sex, age, weight, height, blood group, body mass index (BMI), blood volume, blood pressure, blood count, total

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**Table I** - *Characteristics of the plasma donors and of the plasma units included in the study*

| Characteristics | |
|---|---|
| **Total measured units** | 296 |
| Units with 700 g weight | 215 |
| Units with at least 600 g weight | 282 |
| **Donors** | |
| Males, n (%) | 146 (67.9) |
| Females, n (%) | 69 (32.1) |
| Age (years) (mean SD) | 49.6 (9.4) |
| BMI (kg/m$^2$) (mean SD) | 26.1 (3.6) |
| Blood pressure systolic (mean SD) | 124.0 (9.2) |
| Blood pressure diastolic (mean SD) | 79.1 (5.7) |
| **Blood** | **Mean (SD)** |
| Total cholesterol (mg/dL) | 198.9 (32.9) |
| HDL (mg/dL) | 54.6 (11.6) |
| LDL (mg/dL) | 124.2 (30.2) |
| Triglycerides (mg/dL) | 100.9 (52.2) |
| Glycaemia (mg/dL) | 89.9 (14.0) |
| Creatinine (mg/dL) | 0.87 (0.14) |
| Total proteins (g/dL) | 6.9 (0.4) |
| WBC (10$^3$/µL) | 5.7 (1.5) |
| RBC (10$^6$/µL) | 4.8 (0.4) |
| MCV (fL) | 90.1 (4.1) |
| MCH (pg) | 30.9 (1.9) |
| MCHC (g/dL) | 34.3 (1.2) |
| Haematocrit (%) | 42.8 (2.8) |
| Haemoglobin (g/dL) | 14.6 (1.0) |
| Platelets (10$^3$µL) | 224 (41.3) |
| MPV (fL) | 7.9 (1.3) |
| **Process** | |
| Total volume of the final unit (mL) (median range) | 682 (679 - 700) |
| Total volume of processed blood (mL) (mean SD) | 1,815 (96.5) |
| Number of cycles (mean SD) | 4.1 (0.3) |
| Duration of the process (minutes) (mean SD) | 48.5 (6.0) |
| Volume of ACD-A in the unit (mL) (mean SD) | 90.9 (21.1) |
| Density (g/cm$^3$) (mean SD) | 1.0157 (0.0025) |
| Plasma in the final unit (mL) (mean SD) | 592.5 (21.6) |
| Citrate measured with $^1$H-NMR (mmol/L) (mean SD) | 4.8 (1.06) |
| Volume of ACD-A in the final unit (mL) (mean SD) | 90.9 (21.1) |
| Units with ≥ 600 mL net plasma (%) | 31.6% |
| ACD-A reinfused to the donor (mL) (mean SD) | 60.4 (22.7) |
| Total ACD-A used (mL) (mean SD) | 151.2 (8.2) |
| Plasma in the unit/ACD-A (mean SD) | 8.0 (2.8) |

cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglycerides, glucose, creatinine, ALT/AST transaminase, total proteins, and ferritin.

### Sample size

This study was not designed to test any formal hypothesis. Sample size was determined in order to have sufficient precision in describing the distribution of differences between theoretical ACD-A estimates, i.e. those obtained with the manufacturer's algorithm, and $^1$H-NMR measurements. Further details on sample size and study power are described in Vurro *et al.*[5].

### Statistical analysis

Donors and plasmapheresis procedure characteristics are shown as mean ± standard deviation (SD) or proportions.

To achieve the first aim, we included only the 215 samples (of the 305 total samples collected) that actually reached the target final weight set at 700 g, practically defined as units weighting ≥690 g. To build a predictive model, we considered the 282 samples (of the 305) that reached a final weight of at least 600 g in order to increase variability in final volume collected; procedures that were interrupted for external reasons or for clinical problems were not included.

### Candidate variables for tailoring plasmapheresis

Only variables (**Table I**) that were associated with the final plasma contained in the unit were considered the putative determinants to be included in a linear regression model. Among the physiologically correlated variables (haematocrit, haemoglobin and total proteins), one was chosen to be included in the model according to statistical (strength of association) and practical (the easiest to measure in real practice) criteria.

Finally, we estimated the minimum final volume of the unit in order to have 50%, 80% or 90% probability of having at least 600 mL of net plasma and how this limit changed according to the donor characteristics. We built centile regressions including the final plasma in the unit as the dependent variable and the total volume of the unit as independent variables; the variables resulted as independent predictors in the previous analysis[6]. Here we present the graphs of how the final volume of the unit

n: number; SD: Standard Deviation; BMI: Body Mass Index; HDL: high-density lipoprotein; LDL: low-density lipoprotein; WBC: white blood cell; RBC: red blood cell; MCV: mean corpuscular volume; MCH: mean corpuscular haemoglobin; MCHC: mean corpuscular haemoglobin concentration; MPV: mean platelet volume; ACD-A: anticoagulant citrate dextrose solution A; $^1$H-NMR: proton nuclear magnetic resonance.deviation.

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should be set to obtain 50%, 80% or 90% probability of having at least 600 mL net plasma when varying one of the determinants and setting all the other variables at the mean value.

## RESULTS

We sampled 305 plasma units, in 9 of which the $^{1}$H-NMR spectra could not be quantified, properly due to a poor baseline. **Table I** shows the characteristics of the plasma donors included in the study, as well as the procedure-related parameters.

The machine setting at 700 g implied the collection of approximately 680 mL final volume (**Table I**).

### Determinants of ACD-A volume in plasma unit

For this analysis, we included the 215 samples that reached a final weight of approximately 700 g. The ACD-A volume in each plasma unit was associated with haemoglobin (HGB), haematocrit, total proteins, triglycerides, systolic blood pressure, BMI and HDL; for the last two, it is plausible that the association was due to chance.

In multivariate models, the association with ACD-A volume remained only for HGB, total proteins, and, to a lesser extent, for triglycerides (**Table II**).

We built similar models limiting the analyses to procedures reaching the target, or only to those where donors were

**Table II** - *Association between anticoagulant citrate dextrose solution A (ACD-A) volume and co-variates*

| Characteristics | | | | | | |
|---|---|---|---|---|---|---|
| **Donors** | **beta** | **SE** | **t** | **p-value** | **95%CI** | |
| Sex (females *vs* males) | 0.079 | 0.13 | 0.63 | 0.531 | −0.17 | 0.33 |
| Age | −0.001 | 0.01 | −0.20 | 0.839 | −0.01 | 0.01 |
| Body Mass Index (BMI) (kg/m$^2$) | **0.028** | **0.02** | **1.64** | **0.102** | **−0.01** | **0.06** |
| Blood pressure systolic | **0.011** | **0.01** | **1.56** | **0.119** | **0.00** | **0.02** |
| Blood pressure diastolic | −0.005 | 0.01 | −0.49 | 0.623 | −0.03 | 0.02 |
| **Blood** | | | | | | |
| Total cholesterol (mg/dL) | 0.002 | 0.00 | 1.23 | 0.221 | 0.00 | 0.01 |
| HDL (mg/dL) | **−0.010** | **0.01** | **−1.85** | **0.066** | **−0.02** | **0.00** |
| LDL (mg/dL) | 0.002 | 0.00 | 1.12 | 0.263 | 0.00 | 0.01 |
| Triglycerides (mg/dL) | **0.004** | **0.00** | **2.90** | **0.004** | **0.00** | **0.01** |
| Glycaemia (mg/dL) | −0.001 | 0.00 | −0.24 | 0.807 | −0.01 | 0.01 |
| Creatinine (mg/dL) | **0.728** | **0.43** | **1.68** | **0.095** | **−0.13** | **1.58** |
| Total proteins (g/dL) | **0.573** | **0.15** | **3.71** | **0.000** | **0.27** | **0.88** |
| WBC (10$^3$/µL) | −0.007 | 0.04 | −0.18 | 0.854 | −0.09 | 0.07 |
| RBC (10$^6$/µL) | 0.007 | 0.02 | 0.33 | 0.742 | −0.04 | 0.05 |
| MCV (fL) | **−0.021** | **0.01** | **−1.48** | **0.141** | **−0.05** | **0.01** |
| MCH (pg) | 0.015 | 0.03 | 0.49 | 0.628 | −0.05 | 0.08 |
| MCHC (g/dL) | **0.117** | **0.05** | **2.25** | **0.025** | **0.01** | **0.22** |
| Haematocrit (%) | **0.079** | **0.02** | **3.82** | **0.000** | **0.04** | **0.12** |
| Haemoglobin (g/dL) | **0.279** | **0.06** | **4.84** | **0.000** | **0.17** | **0.39** |
| Platelets (10$^3$µL) | 0.000 | 0.00 | 0.01 | 0.995 | 0.00 | 0.00 |
| MPV (fL) | 0.017 | 0.05 | 0.36 | 0.717 | −0.07 | 0.11 |
| **Process** | | | | | | |
| Plasma density (g/cm$^3$) | 281.245 | 21.73 | 12.94 | 0.000 | 238.48 | 324.01 |
| Total volume of the plasma unit (mL) | −0.003 | 0.00 | −0.74 | 0.458 | −0.01 | 0.01 |
| Total volume of processed blood (mL) | 0.001 | 0.00 | 0.97 | 0.332 | 0.00 | 0.00 |
| N. of cycles | 0.301 | 0.18 | 1.63 | 0.104 | −0.06 | 0.66 |
| Length of the process (minutes) | −0.001 | 0.01 | −0.13 | 0.897 | −0.02 | 0.02 |

Bold numbers are associated with the important co-variates. For this analysis, only 215 units reaching the setting weight of 700 g were included.
BMI: Body Mass Index; HDL: high-density lipoprotein; LDL: low-density lipoprotein; WBC: white blood cell; RBC: red blood cell; MCV: mean corpuscular volume; MCH: mean corpuscular haemoglobin; MCHC: mean corpuscular haemoglobin concentration; MPV: mean platelet volume; N: number.

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rehydrated during the procedure, but uncertainty of estimates was higher compared to all the procedures (with and without rehydration during the process).

### Tailoring the target volume

In this analysis, we included 282 units weighing at least 600 g. Quantile regression allowed us to predict the total plasma volume that should be collected in order to have 50%, 80% or 90% of units with at least 600 mL net plasma volume. **Figure 1** shows how this volume varied according to changes in HGB, total protein and triglycerides. Fixing the other two variables at the average value, an 80% probability of having at least 600 mL net plasma can be reached by setting the machine at 690 g for donors with low HGB levels, while more than 720 g is necessary for donors with high HGB levels. Concerning total proteins, 700 g would be needed in order to reach 80% of units with at least 600 mL net plasma for donors with low protein values, and more than 720 g for those with high protein values. Instead, the weight to be reached to have at least 80% units with 600 mL net plasma is 710 g with low triglycerides and 720 g with high triglycerides.

The model predicts that a 90% certainty of having at least 600 mL net plasma can be reached only by increasing the final weight to values over 725 g for high total proteins, triglycerides or HGB (**Figure 1**).

## DISCUSSION

We built a statistical model based on donor characteristics to predict the amount of net plasma volume obtained with a plasmapheresis procedure. We found that the most predictive parameters were HGB and, to a lesser extent, triglycerides and total proteins. Our model predicts a modest but not negligible opportunity to personalise the machine setting according to these donor characteristics; we can change the final weight of the unit in a range of 20 g. Furthermore, our model predicts that, in most cases, achieving a 90% probability of having at least 600 mL of net plasma in the unit requires collecting very large volumes.

We recently observed an often large discrepancy between ACD-A estimates (based on manufacturers' algorithms) and an exact amount of ACD-A in the plasma unit. This discrepancy is also reflected in our model: using estimated ACD-A, the predictive model does not make any sense since the predicted final variability is minimal and completely dependent on HGB (*Online Supplementary Content*, **Figure S1**).

In our study, the plasmapheresis procedures were set at 700 g in the vast majority of cases, and all units ranged in weight from 600 to 700 g. This limits the model validity because we are extrapolating predictions out of the observed range.

Regarding untraced procedure characteristics, we were mostly concerned with rehydration during the procedure for two reasons: 1) the criteria adopted by the medical staff are subjective and related to individual donors; 2) plasma collection was conducted mostly in the summer, when the weather is often very hot (usually 30-35°C). However, sensitivity analysis excluding the plasma units collected when rehydration occurred during the procedure, showed similar results.

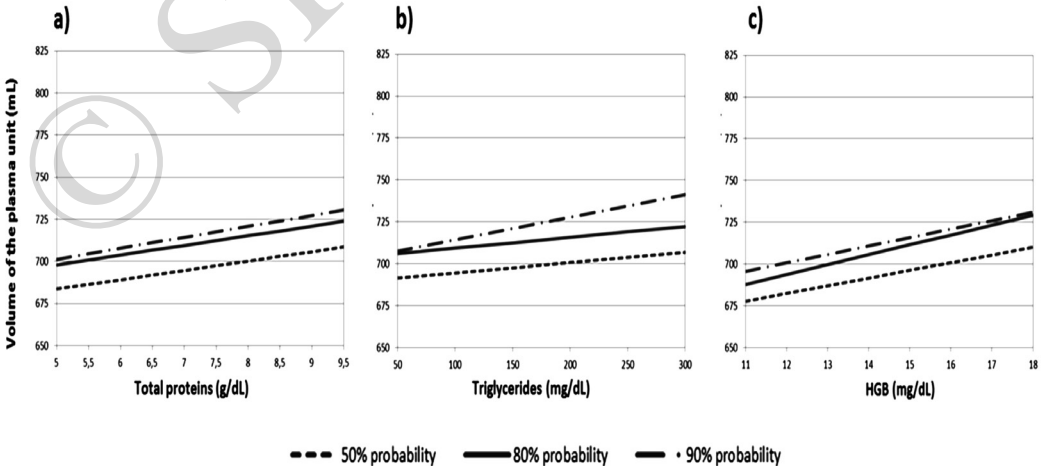



**Figure 1 - Model for predicting the total plasma volume to collect in order to have 50%, 80% or 90% probability of having at least 600 mL net plasma in each unit, according to donor characteristics: total proteins (A); triglycerides (B); haemoglobin (HGB, C)**
The 282 units with final weight over 600 g were included in the analysis. nufacturing flow of cord blood platelet gel (CBPG) for clinical applications.

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Results of our model cannot be generalised because our study is monocentric and focused only on one plasmapheresis machine manufacturer; calculations must be locally adjusted and tailored to the characteristics of the donor population, procedure settings, and type of plasmapheresis machine used.

Nevertheless, we have shown that procedure customisation is possible: a predictive model based on easily measurable donor characteristics (mainly HGB) makes it possible to collect 30 mL less plasma (**Figure 1**) in donors with low HGB levels, or protein and triglycerides. This decrease corresponds to a difference in processed peripheral blood of approximately 100 mL, which can have an impact on the incidence of AEs. As Italian plasma donors are unremunerated volunteers, every attempt must be made to reduce AEs, not only to protect the donors' safety but also to encourage their continued loyalty[7-9]. Nevertheless, we recommend using an analytical method for quantifying ACD-A content in the unit to obtain a reliable prediction rather than relying on ACD-A estimates.

A final aspect that deserves deeper investigation regards the amount of net ACD-A in the final unit; the importance of this can be seen when we consider plasma destined for industrial use to obtain obtain coagulation factors and total proteins[10] or plasma directly destined to recipient patients.

## CONCLUSIONS

Here we have shown that it is possible to predict the probability of collecting a fixed amount of net plasma. Our study paves the way to implementing a donor-centred blood management approach to plasma donation, tailoring procedures on the basis of a few, easily collectable donor characteristics.

Further studies are necessary to verify whether tailored plasma donation could reduce AEs in donors and improve standardisation of the ACD-A content in the plasma used for clinical practice.

Future studies with a donor-centred blood management approach could also identify the "ideal" plasma donor in terms of efficiency, i.e. the collection of at least 600 mL of plasma net of the anticoagulant with the machine calibrated to the lowest product weight.

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## AUTHORSHIP CONTRIBUTIONS

LM designed the study, collected plasma samples, and performed data analysis. CM performed data analysis, wrote, and submitted the manuscript. FV performed nuclear magentic resonance quantitative measures of the anticoagulant. MP performed the plasmapheresis procedures. EdB recruited plasma donors. LB and SB built the predictive model. PGR helped build the predictive model and contributed to preparation of the manuscript. RB designed the study, and contributed to preparation of the manuscript. TAP designed the study, and contributed to data analysis and manuscript preparation.

*The Authors declare no conflicts of interest.*

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