



# Total platelet donation count and donation frequency are determinants of plateletpheresis-associated lymphopenia

Linda Thuer<sup>1</sup> | Andreas Brosig<sup>1</sup> | James A. Hutchinson<sup>2</sup> | Viola Hähnel<sup>1</sup>  | Robert Offner<sup>1</sup> | Ralph Burkhardt<sup>1</sup> | Norbert Ahrens<sup>1,3</sup> 

<sup>1</sup>Institute for Clinical Chemistry and Laboratory Medicine, University Hospital Regensburg, Regensburg, Germany

<sup>2</sup>Department of Surgery, University Hospital Regensburg, Regensburg, Germany

<sup>3</sup>MVZ for Laboratory Diagnostics Raubling, Amedes, Raubling, Germany

## Correspondence

Norbert Ahrens, MVZ for Laboratory Diagnostics Raubling, Amedes, Raubling, Germany.

Email: norbert.ahrens@ukr.de

## Abstract

**Background:** Plateletpheresis using a leukocyte reduction system (LRS) traps donor WBCs in the LRS chamber, which may lead to lymphopenia, especially in frequent plateletpheresis donors. It seems plausible that this might cause adverse effects. However, current knowledge about potential confounders and donor health impacts is incomplete.

**Donors and methods:** Recent platelet donors and donations collected at University Hospital Regensburg from 2016 to 2019 using the Terumo BCT Trima Accel LRS system were retrospectively analyzed and compared with historical platelet donors and donations collected mainly with Fresenius Kabi Amicus non-LRS system from 2010 to 2013. Additionally, recent donors were prospectively surveyed using a health-related topics questionnaire.

**Results:** Analysis of 819 recent donors with 11,254 blood counts and 1464 questionnaires and 1011 historical donors with 12,848 blood counts revealed that increased annual platelet donation frequencies were associated with decreased lymphocyte counts in both groups. Median lymphocyte counts in recent donors with no versus  $\geq 24$  previous annual donations declined from 2.0 to  $1.2 \times 10^3/\mu\text{L}$  ( $p < 2.2 \times 10^{-16}$ ), and those in historical donors with no versus  $\geq 24$  previous annual donations decreased from 2.0 to  $1.5 \times 10^3/\mu\text{L}$  ( $p = 6 \times 10^{-4}$ ), respectively. The questionnaire results showed that donation frequency and lymphopenia were not associated with upper respiratory tract infection (URTI) incidence or duration, but platelet donors who concomitantly donated granulocytes had significantly shorter URTI durations than those who did not ( $p = .008$ ).

**Conclusion:** This study confirmed that plateletpheresis-associated lymphopenia occurs in LRS and to a lesser degree in non-LRS platelet donors, but revealed no evidence of a negative impact on donor health.

## KEYWORDS

blood donation, donation side effects, leukopenia, lymphopenia, platelet donation, plateletpheresis

## 1 | INTRODUCTION

Platelet concentrates are obtained by two methods: by pooling remainders from several whole blood donations or by collecting platelets from a single donor by apheresis. The advantage of the latter method is that it selectively removes platelets and returns the remaining blood components to the donor. Consequently, repeated platelet donations can be collected at short intervals.<sup>1</sup> In the European Union, platelet concentrates are leukoreduced to  $<1 \times 10^6$  white blood cells (WBCs) per unit.<sup>2</sup> The filtered WBCs are either returned to the donor or trapped, when using Terumo's Trima, within a so-called leukoreduction system (LRS) chamber. After platelet harvesting, the trapped WBCs are flushed out of the LRS chamber (generally incompletely) and discarded or donated to research. About  $1 \times 10^9$  WBCs can be obtained from one LRS.<sup>3, 4</sup> This has led to the theory that leukocyte trapping may result in leukopenia in plateletpheresis donors.

There are numerous indications that leukopenia and lymphopenia are independent risk factors for donor health impairment. Recently, large cohort studies have shown that lymphopenia ( $\leq 1.5 \times 10^3$  lymphocytes/ $\mu\text{L}$ ) in outpatients is associated with an increased risk of cardiovascular and non-cardiovascular mortality, independent of age.<sup>5</sup> Moreover, lymphocyte counts were found to decrease with aging.<sup>6</sup> Another study revealed that lymphopenia of  $<1.1 \times 10^3/\mu\text{L}$  in the general population is associated with an increased risk of a range of different infectious diseases and infection-related death, independent of age.<sup>7</sup> Likewise, a recent analysis of 1,390,801 plasma donations and 111,458 LRS and non-LRS plateletpheresis donations revealed that lymphopenia in frequent plateletpheresis donors with high total donation counts was associated with an increased risk of immunosuppression-related infections and common bacterial infections.<sup>8</sup>

Lymphopenia from platelet donation was found to occur with some of the earlier donation techniques used in the 1980s. In one study, a 20% decrease in lymphocyte counts was observed after 10 weekly platelet donations prepared using the Haemonetics Model 30, which removes as much as 30% of the donor's circulating lymphocytes per donation.<sup>9</sup> The introduction of leukodepleted blood products and newer apheresis technologies has reduced but not eliminated this problem. Lymphocyte count decreases of 60/ $\mu\text{L}$  after four platelet donations on with Trima Accel have been observed,<sup>10</sup> one of the most widely used apheresis systems today. Plateletpheresis-associated lymphopenia was recently attributed to low  $\text{CD4}^+$  T-cell counts in frequent donors with a high annual donation frequency on the Trima Accel.<sup>11</sup>

Protecting healthy donors from harm arising from the donation procedure is one of the foremost duties of blood donation services. Therefore, it is important to know if there is a relationship between plateletpheresis-associated lymphopenia and the annual donation frequency, total donation count, concomitant donation of other blood components, and/or the type of plateletpheresis system (e.g., with or without an LRS chamber), and whether plateletpheresis-associated lymphopenia is clinically relevant. Therefore, we performed a retrospective multifactorial analysis of clinical, demographic, and hematological data on recent (with LRS) versus historical (mostly non-LRS) plateletpheresis donors and donations and a prospective survey of leukopenia and health-related aspects in recent donors.

## 2 | DONORS AND METHODS

All eligible plateletpheresis donors processed by the local blood donation service of the University Hospital Regensburg, Germany from 01/01/2010 to 12/31/2013, whose donations were collected mainly without an LRS chamber (historical donors) and those processed from 01/01/2016 to 12/31/2019, whose donations were collected with an LRS chamber (recent donors), were assessed for eligibility in the retrospective analysis of donor and donation-specific data, including hematological variables and donation type (platelets alone or with granulocytes and/or mononuclear cells). However, blood counts from donors mobilized for granulocyte donation were excluded. The time range for the total platelet donation count was from 2005 to 2019.

Additionally, all recent platelet donors presenting at our blood donation service from March 19 to November 29, 2019 were asked to complete a prospective questionnaire on health-related topics during each visit. The questionnaire included questions regarding their health habits (e.g., physical activity and exercise), exposure to environments associated with an increased risk of upper respiratory tract infection (e.g., kindergartens, crowded public areas, and public transport), sleep habits, and infectious disease history as well as a personal "Cold diary" and is provided translated in the Data S1. The questionnaire was focused on respiratory tract infections, as these were common in the pre-Corona era.

A total of 1531 questionnaires were submitted, 48 of which had to be excluded (10 due to missing consent, 18 due to missing donation identification, and 20 due to questionnaire submission without qualification for donation). After the remaining 1483 eligible questionnaires were read into an electronic data processing system (EvaSys v8.0, Lüneburg, Germany), another 74 were

excluded—two from mononuclear cell (MNC) donors and 72 from donors who presented without donation. Thus, 1409 out of 1642 platelet donations collected during the questionnaire survey period were included from 320 donors in the analysis, corresponding to a participation rate of 86%.

The study was approved by the independent ethics committee of the University of Regensburg (approval # 19-1348-101).

## 2.1 | Apheresis procedures

Plateletpheresis was performed using the non-LRS Amicus Cell Separation Platform (Fresenius Kabi) in 73% of donations obtained in 2010–2013 (with the remainder on LRS-based donations), and with the LRS-based Trima Accel Automated Blood Collection System (Terumo BCT) in 99% of donations from 2016 to 2019. Generally, double and single plateletpheresis products were collected from all donors with platelet counts above 200/nL and 150/nL, respectively, according to local and EU legislative regulations.<sup>2</sup> A maximum of 26 annual plateletpheresis was allowed. The Trima Accel software offers no option to flush the leukoreduction chamber apart from the rinseback of the tubing system after platelet harvesting.

Mononuclear cell collection was performed using the Cobe Spectra Auto-PBSC protocol (Terumo BCT) until 2014, and the Spectra Optia Continuous Mononuclear Cell Collection (cMNC) program (Terumo BCT) thereafter, as described previously for extracorporeal photopheresis.<sup>12</sup>

Granulocytapheresis was done as previously described with the Cobe Spectra MNC program using hydroxyethyl starch as the red blood cell (RBC) sedimentation enhancer until 2014, and with the Optia cMNC program using modified fluid gelatin to enhance RBC sedimentation from then on.<sup>13</sup>

## 2.2 | Hematological analysis

Hematological parameters, including hemoglobin (Hb), WBC, lymphocyte, monocyte, neutrophil, and platelet counts, were routinely measured before each plateletpheresis donation. This was done using the Sysmex XE-5000 Automated Hematology Analyzer until 2016 and the Sysmex XN550 in the years thereafter.

## 2.3 | Statistical analysis

Donation history and hematological data were extracted from the laboratory information system (Swisslab, Berlin,

Germany) and from the hospital's electronic data system (SAP) and merged electronically with the questionnaire data using R. Statistical analysis and graphical presentation of the data were performed in the R software environment using the packages *tidyr* and *dplyr*, *openxlsx*, *reshape2*, *psych*, *nortest*, *data.table*, *gplots*, *rpart*, *rpart.plot*, *randomForestSRC*, *ggRandomForests*, *ggrepel*, and *ggplot2*'s *cowplot* as well as *RColorBrewer* and *scales* along with their dependencies and is publicly available.<sup>14</sup> Normal distribution was not found in any of the tested data, as determined by Levene's test for equality of variances. Therefore, significance testing was performed with Spearman's rank correlation coefficient, Wilcoxon, and/or Kruskal-Wallis test, as appropriate. Factor analysis was subsequently performed using the Kaiser–Meyer–Olkin (KMO) test.

## 3 | RESULTS

### 3.1 | Recent donors (LRS donations)

The group of recent donors, who mainly underwent plateletpheresis with an LRS chamber, comprised 819 donors (467 male, 352 female) with 11,254 blood counts, 98.7% of which were differential counts (Table 1). Some of these had additional granulocyte (65) or mononuclear cell donations (42), or both (40) at least once. The median number of platelet donations was higher in men than in women, both annually ( $\sigma = 6.2$ ,  $\varphi = 5.1$ ) and overall ( $\sigma = 9$ ,  $\varphi = 7$ ). Likewise, the annual platelet donation frequency in older donors aged 30 and older (five per year) was higher than that in younger donors below the age of 30 (two per year;  $p = 1.7 \times 10^{-14}$ ).

The association between donation frequency and lymphopenia was highly significant, as expressed by a rho of  $-0.41$  ( $p < 2.2 \times 10^{-16}$ , Figure 1a). Donors with at least 24 plateletpheresis donations per 365-day period had a median lymphocyte count of  $1.24 \times 10^3/\mu\text{L}$  compared with  $2.02 \times 10^3/\mu\text{L}$  in donors without prior plateletpheresis. Neutrophil and monocyte counts showed minor dependency on the donation frequency: rho values were  $-0.05$  ( $p = 4.325 \times 10^{-8}$ ) and  $0.09$  ( $p < 2.2 \times 10^{-16}$ ), respectively (Figure 1a).

Lymphopenia was also associated with the total platelet donation count, as expressed by a rho of  $-0.45$  ( $p < 2.2 \times 10^{-16}$ , Figure 1b). Associations for neutrophils and monocytes were less pronounced, as reflected by a rho  $-0.05$  ( $p = 4.3 \times 10^{-8}$ ) and  $0.09$  ( $p < 2.2 \times 10^{-16}$ ), respectively (Figure 1b).

The annual platelet donation frequency and total platelet donation count (cumulative) were highly associated with each other, and both were associated with

TABLE 1 Donor characteristics

	Male	Female
<b>Historical group of mostly non-LRS plateletpheresis donors (01/01/2010–12/31/2013)</b>		
Number of donors ( $n = 1011$ )	565	446
Hematological test visits ( $n = 12,848$ )	7987	4861
Fraction with differential cell counts (%)	12.6	10.9
Sex distribution (%)		
Donors	55.9	44.1
Visits	61.4	38.6
Age (years)		
Median age	26.0 (18.6–58.9)	26.0 (18.3–57.8)
Age at donation	27.6 (18.3–60.7)	28.9 (18.2–59.0)
Platelet donations ( $n$ )		
Total	10 (0–148)	7 (0–103)
Annual	6.7 (0–33.2)	5.1 (0–28.1)
Concomitant MNC donations ( $n$ )		
Total	0 (0–6)	0 (0–0)
Annual	0 (0–1.8)	0 (0–2.0)
Concomitant granulocyte donations ( $n$ )		
Total	0 (0–10)	0 (0–0)
Annual	0 (0–7.9)	0 (0–3.0)
Hematological parameters		
Hb [g/dL]	15.1 (12.8–18.05)	13.2 (11–15.9)
WBC [ $\times 10^3/\mu\text{L}$ ]	5.80 (3.42–12.78)	6.32 (3.26–11.06)
Lymphocytes [ $\times 10^3/\mu\text{L}$ ]	1.88 (0.72–3.90)	2.04 (0.79–4.12)
Monocytes [ $\times 10^3/\mu\text{L}$ ]	0.54 (0.24–1.32)	0.48 (0.25–1.25)
Neutrophils [ $\times 10^3/\mu\text{L}$ ]	3.21 (1.39–7.65)	3.45 (1.25–8.95)
Platelets [ $\times 10^3/\mu\text{L}$ ]	234 (144–346)	262 (177–411)
<b>Recent group of mostly LRS plateletpheresis donors (01/01/2016–12/31/2019)</b>		
Number of donors ( $n = 819$ )	467	352
Hematological test visits ( $n = 11,254$ )	7391	3863
Fraction with differential cell counts [%]	98.2	99.6
Sex distribution (%)		
Donors	57	43
Visits	65.4	34.6
Age (years)		
Median age	26.3 (18.1–64.8)	25.5 (18.2–57.2)
Age at donation	28.7 (18.1–66.8)	26.7 (18.1–57.2)
Platelet donations ( $n$ )		
Total	9 (0–218)	7 (1–159)
Annual	6.2 (0–28.1)	5.1 (0.1–26.1)
Concomitant MNC donations ( $n$ )		
Total	0 (0–8)	0 (0–0)
Annual	0 (0–2.9)	0 (0–0)

TABLE 1 (Continued)

	Male	Female
Concomitant granulocyte donations ( <i>n</i> )		
Total	0 (0–17)	0 (0–0)
Annual	0 (0–3.4)	0 (0–0)
Hematological parameters		
Hb [g/dL]	15.05 (13.3–17)	13.25 (12.1–15.7)
WBC [ $\times 10^3/\mu\text{L}$ ]	5.71 (3.54–10.86)	6.52 (4.02–12.02)
Lymphocytes [ $\times 10^3/\mu\text{L}$ ]	1.82 (0.78–3.35)	2.14 (0.89–4.66)
Monocytes [ $\times 10^3/\mu\text{L}$ ]	0.54 (0.27–1.28)	0.52 (0.26–0.1)
Neutrophils [ $\times 10^3/\mu\text{L}$ ]	3.12 (1.59–6.89)	3.58 (1.72–8.26)
Platelets [ $\times 10^3/\mu\text{L}$ ]	242 (166–416)	278 (181–420)

Note: Data are shown as median values with range in parentheses. Visits include all initial donor presentations, visits where donors were rejected, and donation visits (including granulocyte donations) at which hematological testing was performed. The annual donation count is shown as a median value calculated based on the donation frequency over the last 365 days.

lymphopenia (Table 2 and Figure 2). Our data also confirm the previously described age-dependent reduction of lymphocyte counts ( $p < 2.2 \times 10^{-16}$ , Figure 3a). Further associations were found for concomitant MNC donations, but not for concomitant granulocyte donations (Figure 3b,c).

### 3.2 | Historical donors (mostly non-LRS donations)

Based on the hypothesis that plateletpheresis-associated lymphopenia is caused by LRS chambers acting as WBC traps, we retrospectively compared hematological parameters and donation frequencies of recent plateletpheresis donors, whose donations were collected using an LRS chamber, with those of the historical donors, whose donations were predominantly collected using the Amicus system, which operates without an LRS chamber. The historical group comprised 1011 donors with 12,848 blood counts, 12.0% of which were differential counts (Table 1). Some of these had additional granulocyte (64) or mononuclear cell donations (58) or both (23) at least once. Platelet donation frequency-dependent lymphopenia also occurred in this historic group, albeit to a weaker extent (Figure 4a). Donors with 24 or more platelet donations in the last 365 days had a median lymphocyte count of  $1.53 \times 10^3/\mu\text{L}$  in peripheral blood, whereas those with zero previous annual plateletpheresis donations had a median of  $1.96 \times 10^3$  lymphocytes/ $\mu\text{L}$  ( $p = .0006$ , Figure 4a,b).

### 3.3 | Multifactorial analysis of recent donors with LRS donations

The different factors causing lymphopenia were dissected in a multifactorial analysis (Figure 5). The Kaiser–

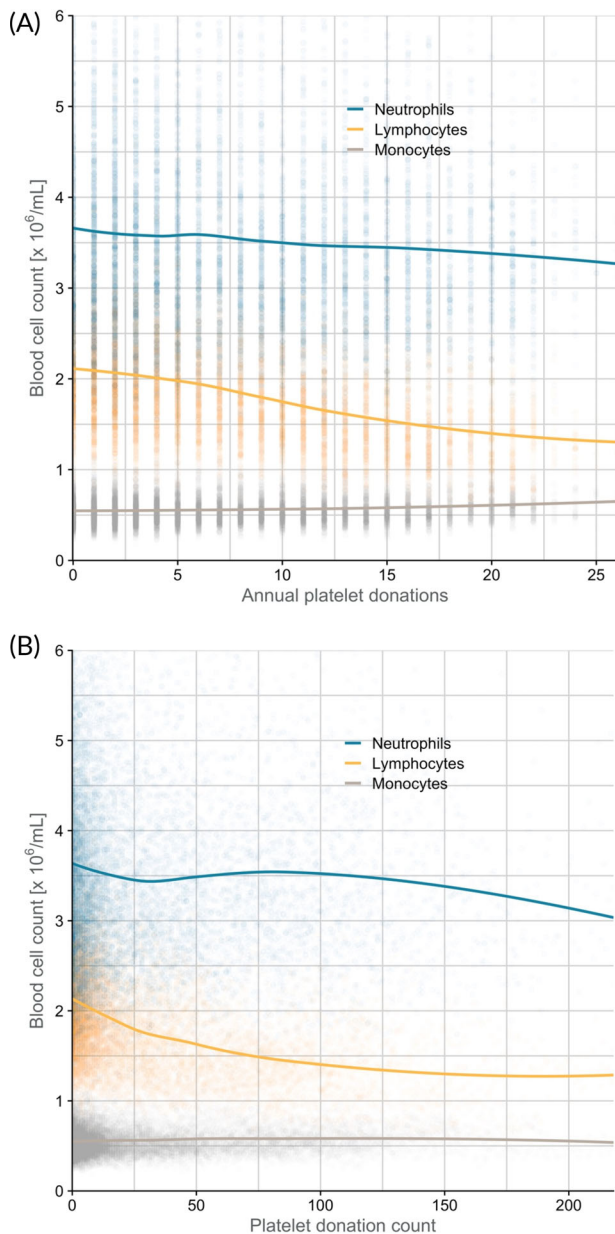
Meyer–Olkin (KMO) test of factorial adequacy revealed that the measure of factoring adequacy was 0.67. KMO values of 0.5 and higher generally indicate the appropriateness of factor analysis. Two-factor maximum-likelihood analysis, which clusters parameters independent of explanatory variables, revealed that Factor 2 (age, lymphocyte count, annual platelet donation frequency, and total platelet donation count) explained 25% of the variance (Figure 5a), while Factor 1 (annual and total number of concomitant MNC and granulocyte donations) explained a further 25% of the variance.

The most important variables for lymphopenia, identified using a tree-based approach computed with the randomForestSRC package (Fast Unified Random Forests for Survival, Regression, and Classification), were total platelet donation count followed by donor age, length of the donor career, and annual platelet donation frequency (Figure 5b).

Dependencies between median lymphocyte values and explanatory variables were calculated with a recursive regression tree model (Figure 5c), which also identified the total platelet donation count as the most important variable. Moreover, the decisive threshold number of plateletpheresis donations for lymphopenia was 29, and the effect was significant ( $p = 0.005$ ). In these two analyses, the age of the donor, the length of the donor career, and the annual platelet donation frequency were identified as other important variables, whereas the number of concomitant MNC and granulocyte donations played a subordinate role (Figure 5b,c).

### 3.4 | Lymphopenia and infections

LRS donors were surveyed about post-donation complications potentially caused by plateletpheresis, such as



**FIGURE 1** Neutrophil (turquoise), lymphocyte (yellow), and monocyte (taupe) counts from 10,762 peripheral blood samples of Trima/LRS platelet donors (dots) and predicted dependencies as a function of annual platelet donation frequency (A) and total platelet donation count (B) with logistic regression by generalized additive models (lines) [Color figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

lymphopenia and upper respiratory tract infection (URTI). Responses to infectious disease items were provided on 1464 of 1483 questionnaires. No infectious diseases were reported in 1307 of 1464 cases (89.3%). Donor lymphocyte counts correlated weakly with the reported number of days with upper respiratory tract infection (“URTI days”, Figure 6a), the number of URTI episodes (“URTI count”, Figure 6c), and the duration of disease

between donations (“URTI duration”, Figure 6e), but these trends were not statistically significant ( $p = 0.16$ ,  $0.16$ , and  $0.22$ , and  $\rho = 0.08$ ,  $0.08$ , and  $0.09$ , respectively).

The platelet donation frequency did not positively correlate with the reported number of URTI days (Figure 6b), URTI episodes (Figure 6d), or the duration of disease between donations (URTI duration, Figure 6f). The results for these aggregated data from 320 donors were not significant ( $p = 0.26$ ,  $0.48$ , and  $0.14$ , and  $\rho = -0.06$ ,  $-0.04$ , and  $-0.12$ , respectively).

As donors with high donation frequencies were included more than once, the data were aggregated as described above. In addition, the donation time span covered by donations was color-coded by the number of days for better visualization (Figure 6a–d). This analysis revealed no obvious distortion in participation days.

In addition, platelet donors with concomitant granulocyte or MNC donations had shorter self-reported URTI durations (median of 3 and 4 days,  $n = 210$  and  $82$ , respectively) than those who donated platelets alone (median of 5 days,  $n = 1261$ ). The difference was statistically significant for granulocyte donations ( $p = 0.008$ ), but not for MNC donations ( $p = 0.5$ ).

Other factors potentially associated with URTI (public transportation, professional contact to groups of children, frequent social contacts, smoking, vitamin supplements, fruit diet, physical exercise, sleep duration, and reported stress) showed no conspicuous effects.

Interestingly, allergies improved more often in frequent donors with a median annual platelet donation frequency of 6.75 compared with those with a median of 2.75 donations per year ( $p = 0.07$ ,  $\rho = 0.10$ ). However, this difference was only detected in a small number of cases ( $n = 15$  of 314).

## 4 | DISCUSSION

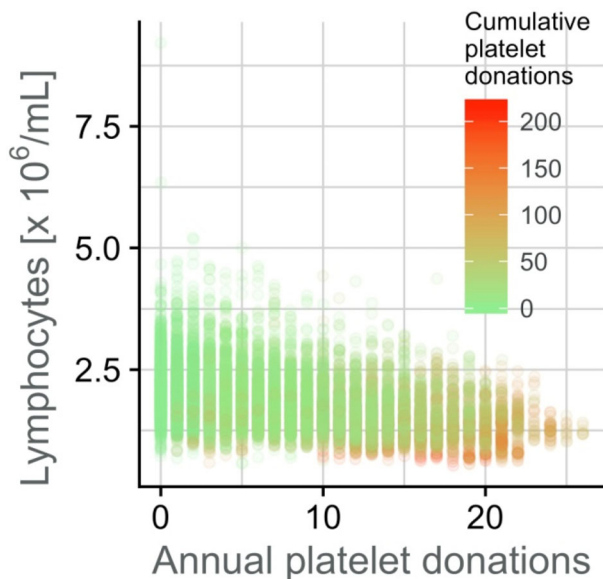
This study shows that platelet donation with the Trima Accel system, which uses an LRS chamber, leads to linearly dose-dependent lymphopenia. This effect varies depending on the type of donation collection system. It was strong and clearly visible in Trima Accel (LRS) donors but weaker in donors whose donations were collected using the Amicus system, which operates without an LRS chamber. The total platelet donation count was identified as the most important factor for plateletpheresis-associated lymphopenia.

Plateletpheresis-associated lymphopenia was a special focus of discussion in the 1980s. As platelet concentrates obtained using older devices such as the Haemonetics 30 contained significant amounts of lymphocytes, donors

**TABLE 2** Changes in hematological variables between the initial and final blood counts

Variable	Initial count	Final count	Change	<i>p</i>
<b>Recent donors (LRS donations)</b>				
WBCs ( $\times 10^3/\mu\text{L}$ )	6.32	6.05	-0.27	$1.74 \times 10^{-8}$
Lymphocytes ( $\times 10^3/\mu\text{L}$ )	2.01	1.86	-0.15	$<2.2 \times 10^{-16}$
Monocytes ( $\times 10^3/\mu\text{L}$ )	0.54	0.53	-0.01	0.004682
Neutrophils ( $\times 10^3/\mu\text{L}$ )	3.46	3.34	-0.12	0.01144
Platelets ( $\times 10^3/\mu\text{L}$ )	255	262	+7	$1.29 \times 10^{-11}$
Hb (g/dL)	14.4	14.3	-0.1	$3.53 \times 10^{-9}$
<b>Historical donors (mostly non-LRS donations)</b>				
WBCs ( $\times 10^3/\mu\text{L}$ )	6.20	6.06	-0.14	0.001098
Lymphocytes ( $\times 10^3/\mu\text{L}$ )	1.95	1.83	-0.12	0.187
Monocytes ( $\times 10^3/\mu\text{L}$ )	0.51	0.52	+0.01	0.8388
Neutrophils ( $\times 10^3/\mu\text{L}$ )	3.44	3.18	-0.26	0.2708
Platelets ( $\times 10^3/\mu\text{L}$ )	249	243	-6	$1.58 \times 10^{-10}$
Hb (g/dL)	14.3	14.2	-0.1	$1.30 \times 10^{-14}$

*Note:* Results are shown as median of hematological values determined before the first and last donation in the study period. Decreases are represented as negative change, and increases as positive change. *p* values were determined by the Wilcoxon signed-rank test. All changes in the recent donors, whose plateletpheresis donations were collected with a leukoreduction system chamber (LRS donations), were significant. Significant declines in WBCs, platelets, and hemoglobin were detected in both groups.



**FIGURE 2** Lymphocyte count as a function of annual platelet donation frequency and cumulative platelet donation count in the group of recent donors with LRS apheresis [Color figure can be viewed at wileyonlinelibrary.com]

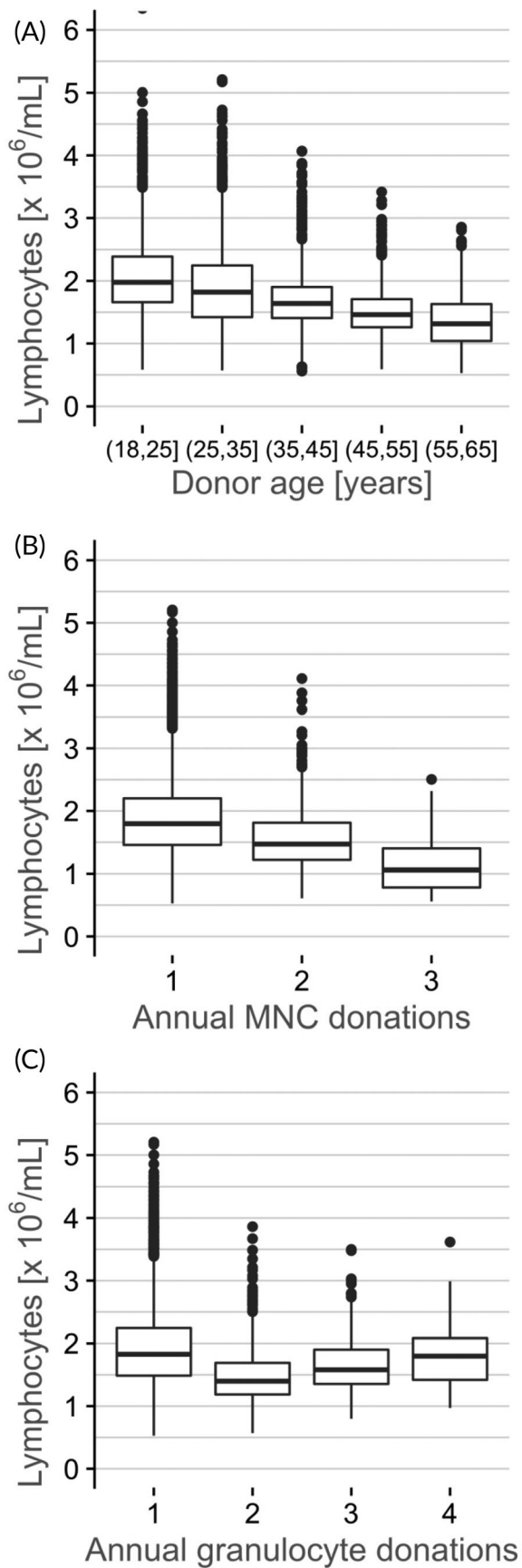
previously lost up to  $5 \times 10^9$  lymphocytes per donation.<sup>15-17</sup> This type of lymphocyte loss was soon found to be dependent on the type of apheresis system. It was reduced from around  $2.8 \times 10^9$  to  $0.6 \times 10^9$  lymphocytes by switching from the Haemonetics 30 device to the Fenwal CS-3000, the precursor of the Amicus system.<sup>18</sup>

The introduction of leukoreduction has substantially reduced donor lymphocyte loss even further. This raises the question of the clinical relevance of plateletpheresis-associated lymphopenia, as no adverse effects were observed, even after several months of lymphopenia.<sup>9, 15, 18-22</sup>

Studies of lymphapheresis therapies for rheumatoid arthritis or thoracic duct drainage involving the removal of up to  $130 \times 10^9$  lymphocytes during the course of treatment revealed no specific susceptibility to bacterial or fungal infections.<sup>20</sup>

Lymphopenia is generally associated with an increased risk of infection,<sup>7, 23</sup> increased mortality,<sup>24-26</sup> and longer hospital stays.<sup>26</sup> This was not recognized in blood donors for many years. These concerns faded as apheresis technologies improved. Eventually, new automated blood separation devices evolved that enabled the production of leukocyte-depleted platelet concentrates with a leukocyte content of less than  $10^6$ , thus significantly reducing lymphocyte losses.<sup>1</sup>

However, a recent analysis of very detailed and complete data from the Swedish portion of the Scandinavian Donations and Transfusions SCANDAT3-S database, providing data on blood donors, donations, components, transfusions, and recipients from the mid-1990s to 2018, revealed an increased risk of common bacterial infections in apheresis donors with more than 50 donations collected with an LRS chamber.<sup>8</sup> As only data on inpatient treatment of post-donation infectious complications are



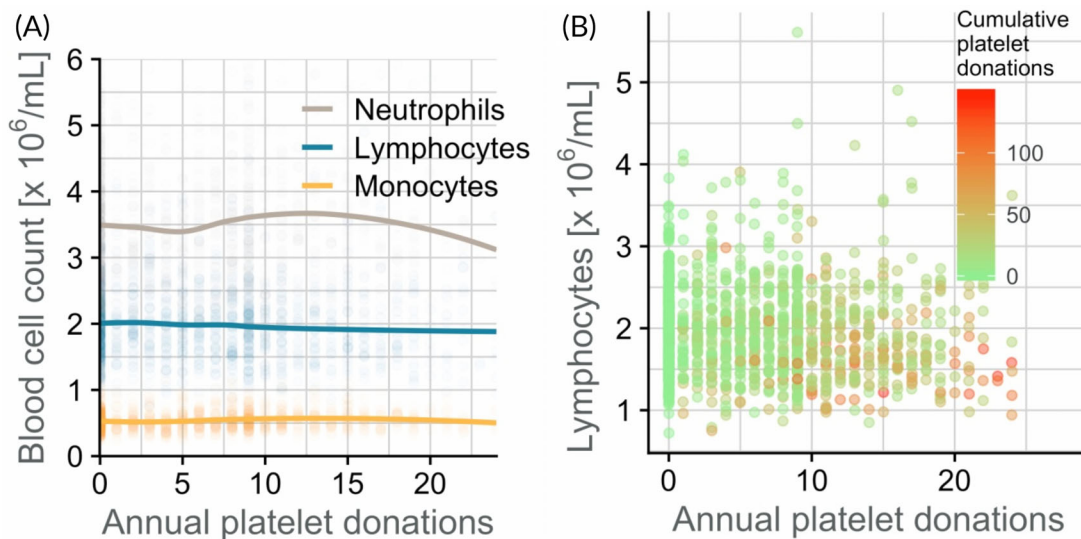
**FIGURE 3** Peripheral predonation blood lymphocyte count as a function of (A) donor age and the number of (B) mononuclear cell (MNC) and (C) granulocyte donations per year in the recent donor group

included in the SCANDAT3-S database, the incidence rates reported in the Swedish study (4.6 events per 1000 person-years among the most frequent donors and 4.1 per 1000 person-years for all donors with >90% LRS donations) are much lower than those in the present study (1372 events per 1000 person-years among LRS donors in our recent data group), which was based on an analysis of donor diary entries. In both cases, the donations were collected before Coronavirus social distancing and face masking requirements. Therefore, although 74,408 donors were included in the Swedish study, the observed association between LRS donations and the risk of infection should be interpreted with caution considering the very small number of events (11 affected LRS donors), the lack of multifactorial analysis to exclude plasma donation bias there in contrast to the present study (plasma donations were 14.7 times more frequent than platelet donations in the Swedish data set), and differences in the population characteristics. Moreover, the proportion of females was 20.1% in the affected LRS donor group compared with 50.5% in the comparison group of the Swedish study.<sup>8</sup>

Concomitant donations of different blood products, as in the Scandinavian study, are common.<sup>8</sup> Some of our donors were likewise granulocyte or mononuclear cell donors. By concept, this would render the donor to a much greater risk of lymphopenia. However, multifactorial analysis failed to prove this effect. Instead, platelet apheresis proved to be the most important factor for lymphopenia.

Our study suggests that the type of cell separator used has an impact on donor lymphocyte loss. While Gansner et al.<sup>11</sup> observed donation frequency-dependent CD4<sup>+</sup> and CD8<sup>+</sup> T-cell lymphopenia in frequent plateletpheresis donors processed with the LRS-based Trima Accel system, no severe CD4<sup>+</sup> T-cell lymphopenia in frequent plateletpheresis donors whose cells were collected with the non-LRS Fenwal Amicus system in another trial.<sup>27</sup> Rhamani et al.,<sup>28</sup> who examined the relationship between lymphocyte counts and plateletpheresis with the LRS-based Trima Accel, also found CD4<sup>+</sup> T-cell lymphopenia in previous frequent apheresis platelet donors who had not donated for at least 1 year. This contradicts the results of Richa et al.,<sup>10</sup> who found no lymphopenia in 471 donors collected with Trima Accel. However, since the median number of platelet donations was only four (range 1–34) and the median number of platelet products donated was seven (range 2–65) over a median of 72 weeks (range 0.3–131.3), and different individual donation frequencies were not differentiated in that study, the validity of its results could be considered limited with regard to long-term frequent donors. The median decrease in lymphocyte count between the first and last donation was 3.7% in this aforementioned study.





**FIGURE 4** (A) Neutrophil (taupe), lymphocyte (turquoise), and monocyte (yellow) counts from 1257 peripheral blood samples from historical donors (dots), whose donations were collected mainly without a leukoreduction system chamber, and predicted dependencies from the donation frequency as generalized additive model (lines), (B) lymphocyte count as a function of annual and cumulative platelet donation rates in the historical group of donors with non-LRS apheresis [Color figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

This is similar to the median decrease in lymphocyte count (7.5%) measured in the present study, but is well below the decrease of 40% found in our frequent donors with  $\geq 26$  apheresis platelet donations per year. This evidence supports the theory that each plateletpheresis donation contributes to a cumulative loss of lymphocytes, which can result in a certain degree of lymphopenia over the long-term in frequent plateletpheresis donors.

The observed lymphocyte loss in plateletpheresis donors may be influenced by the type of WBC reduction technology used during apheresis. While the Amicus system achieves leukocyte reduction based on centrifugation with the elution principle,<sup>29</sup> Trima Accel uses an LRS chamber with the countercurrent principle. Uncollected WBCs are reinfused back to the donor by the Amicus system, but remain in the LRS chamber of the Trima Accel.

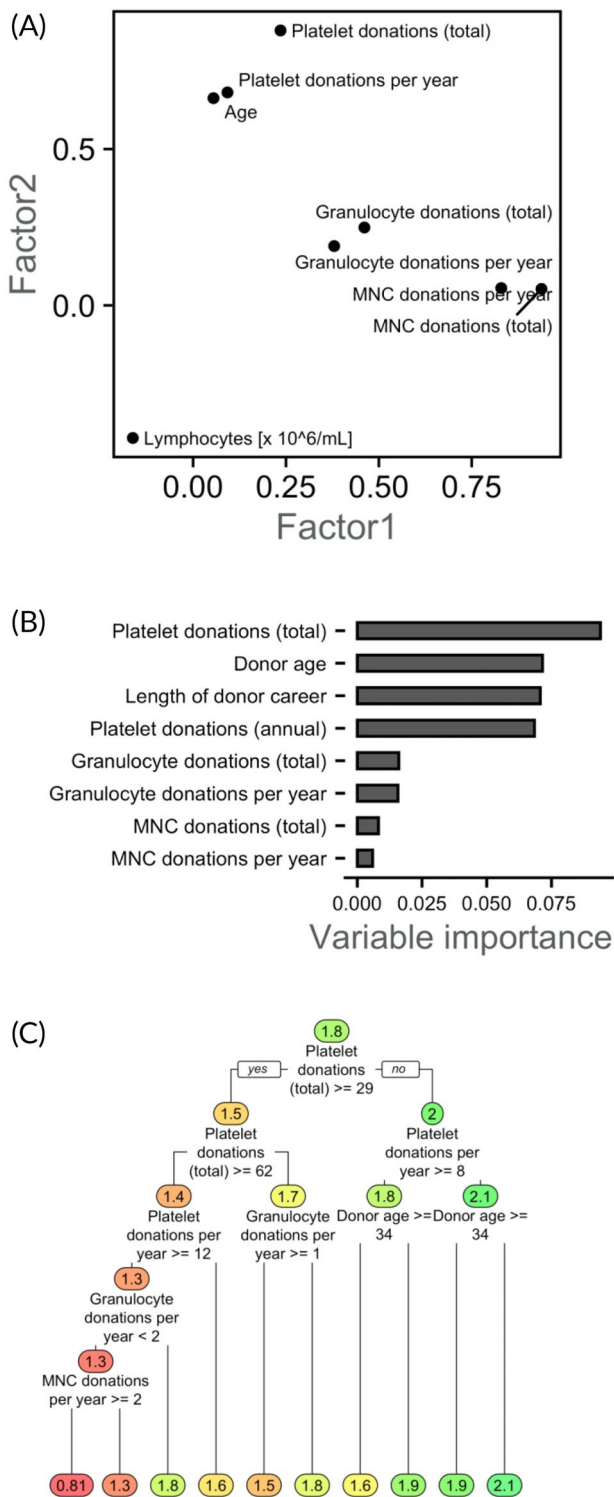
Investigators have calculated different estimates for the lymphocyte content of LRS chambers, which range from about  $0.6$  to  $1.4 \times 10^9$  WBC.<sup>3, 4, 11, 30, 31</sup> This wide variation can be explained by differences in anticoagulant ratios, device settings, draw and return management, processed blood volumes, and annual donation frequencies between studies.<sup>11, 30</sup> One study reports that the LRS chamber caught 15%–20% of mononuclear cells passing through the apheresis device.<sup>32</sup> Accordingly, a rate of 26 donations per year would result in a loss of at least  $16 \times 10^9$  lymphocytes due to the content of lymphocytes trapped in the LRS chambers.

This is a moderate number, as only 2.2% of all human lymphocytes (approximately  $10 \times 10^9$  lymphocytes) circulate in the blood.<sup>33</sup> While reduction of this pool

through apheresis appears evident, and exhaustion of the replenishments would be assumed, other explanations are possible. The circulating lymphocyte pool is not generated by overflow of marrow and lymphoid tissues, where these cells come from, but rather substance of regulated release. Lower levels of circulating lymphocytes after apheresis might therefore also be explained by lowered lymphocyte target values within physiological limits. This would explain why clinical signs of lymphopenia are generally not known as blood donation side effects.

However, lymphopenia does occur after blood donation and is evident by statistical methods. On an individual level, lymphocyte levels ranging from  $1.0$  to  $1.5 \times 10^3/\mu\text{L}$  of blood are classified as lymphopenia.<sup>7</sup> Lymphopenia with less than  $1.1 \times 10^3$  lymphocytes/ $\mu\text{L}$  was observed in 4.0% of frequent donors in our LRS group, whose median annual platelet donation frequency was 12.7 compared with 6.1 in the overall LRS group. Age might have contributed to lymphopenia in frequent LRS donors, as their median age was 38.0 years compared with 25.9 years in the overall LRS group. As donation intensity was not evenly distributed through donor ages, this retrospective analysis does not allow to draw conclusions on age effects.

No negative impact of plateletpheresis-associated lymphopenia on donor health was demonstrated in this study. We found that low lymphocyte counts and a high annual platelet donation frequency did not negatively affect the frequency and duration of upper respiratory tract diseases in recent plateletpheresis donors. Donors



**FIGURE 5** Impact of annual platelet donation frequency, total platelet donation count, concomitant granulocyte donation, and concomitant MNC donation on lymphocyte count. Shown are the results of (A) exploratory maximum-likelihood factor analysis, (B) random forest analysis of the importance of these variables for lymphocyte count, and (C) recursive regression tree analysis. In the latter case, the average group lymphocyte count is shown as  $10^6/\text{mL}$  inside ovals; the left branches indicate cases where the condition is met, and the right branches show where it is not [Color figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

on non-LRS plateletpheresis systems from the historic group were not included in the questionnaire, as these were less affected by lymphopenia, and there was no suspicion of infectious disease complications. In addition, 4.7% of our recent study participants reported an improvement of allergies in connection with plateletpheresis. However, this observation should be interpreted with caution, as only a few donors reported on allergies, and this study did not have enough cases for sufficient statistical power. Thus, pathophysiological hypotheses based on mechanical stress to the cells and/or changes in electrolytes are speculative. In particular, heat shock protein expression by B cells was shown to be upregulated by the mechanical stress of apheresis.<sup>34</sup>

The blood exposure to biomaterials during extracorporeal circulation has a modulating effect on the body's own cells. Some investigators concluded that plateletpheresis does not lead to an increase in platelet activation in donors,<sup>35, 36</sup> while others found that the exposure of blood to any foreign materials during plateletpheresis could potentially result in the activation of blood platelets, coagulation pathways (even if anticoagulants are used), complement system activation and, thus, to leukocyte activation.<sup>37, 38</sup>

According to Huang et al.,<sup>39</sup> the adsorption of plasma proteins to membrane surfaces in the extracorporeal circuit is one of the byproducts of this generalized activation process. Ghio et al.<sup>40, 41</sup> reported that plasma protein adsorption during apheresis leads to the formation of a boundary layer of soluble human leukocyte antigen class 1 (sHLA-I) molecules, to which activated neutrophils and  $\text{CD8}^+$  T lymphocytes could bind and induce multilevel immune modulation—a condition associated with apoptosis and impairment of the immune system.<sup>41, 42</sup> However, in contrast to therapeutic apheresis and dialysis patients, healthy donors exhibited these as short-lived effects, with no increase in the concentration of transforming growth factor-beta-1 ( $\text{TGF}\beta 1$ ) in the plasma.<sup>40, 41, 43</sup> Therefore, Ghio et al.<sup>41, 44</sup> did not find any evidence suggesting that apheresis donors develop any immunosuppressive side effects like bacterial, viral, or neoplastic diseases. The tendency of allergies to decrease after frequent plateletpheresis donations was not mentioned in the literature.

Two positive aspects of the present study are the large sample size of the donor population and the long follow-up period for monitoring the hematological outcome variables. Thirdly, we compared two different groups of donors whose plateletpheresis donations were collected with and without an LRS chamber at the same blood donation center in different time periods. Moreover, the continuous prospective capture of clinical immune status assessment data before each donation by questionnaire is

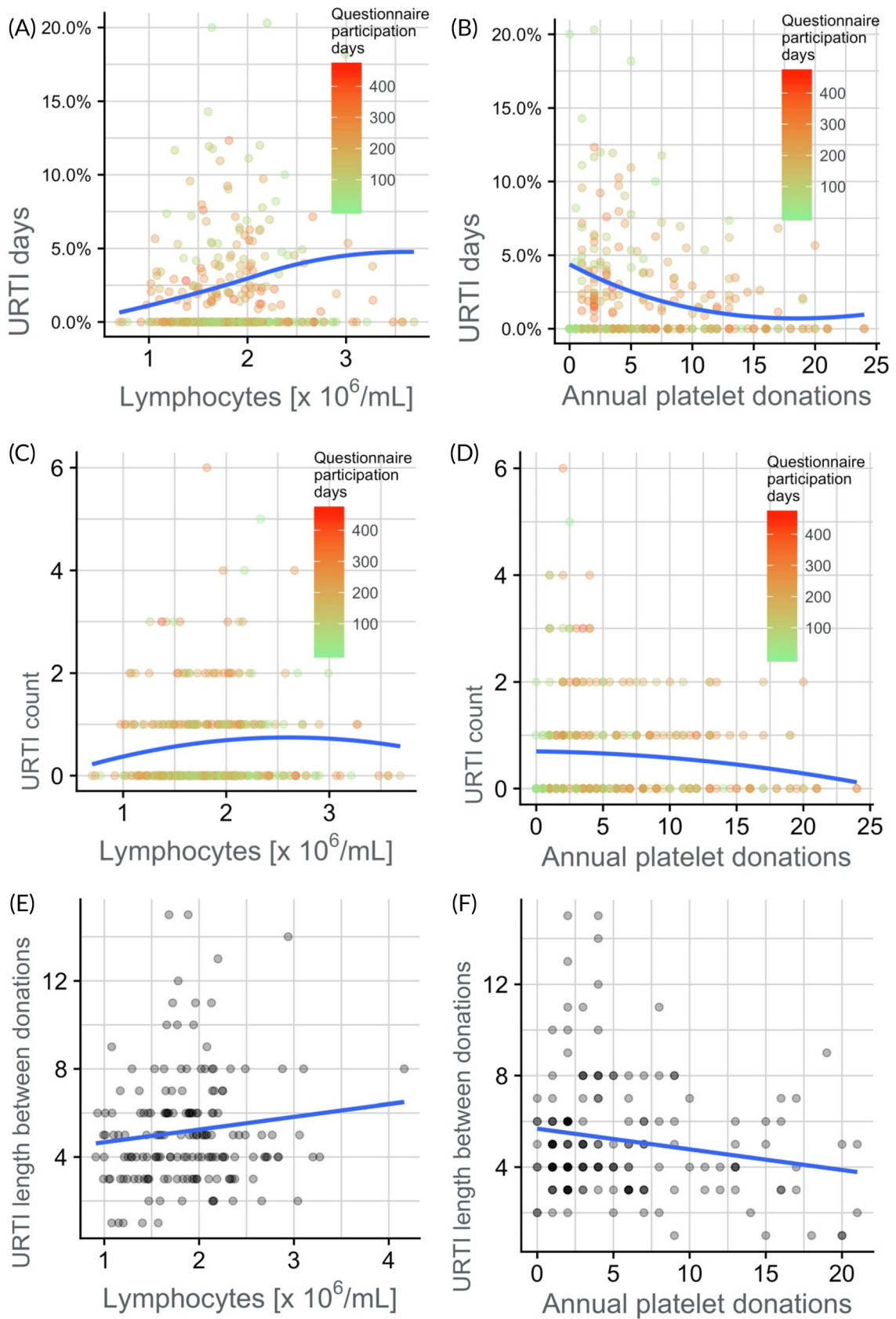


FIGURE 6 Legend on next page.

a feature that distinguishes the present study from others, in spite of the fact that the questionnaire survey period was relatively short and based on donor self-assessments.

However, the donors alone were responsible for maintaining their personal diary of colds and allergies, and there was no non-donor control group. One of the main limitations of the retrospective study design was poor comparability of the historical donors with non-LRS apheresis and recent donors with LRS apheresis because differential blood counts could only be obtained retrospectively. Other limitations include the lack of randomization of donors to LRS and non-LRS apheresis, the lack of lymphopenia severity classification in the various lymphocyte subgroups, and the lack of testing for functional changes in the cells.

## 5 | CONCLUSION

In summary, this study demonstrated a significant association between frequent plateletpheresis using apheresis devices with an LRS chamber and reduced lymphocyte counts, but the duration of this lymphopenia remains unclear. Our findings do not suggest that plateletpheresis-associated lymphopenia harms the donors' immune competence, but the theory that apheresis may induce changes in the immune system remains plausible. Blood donation services may therefore want to ensure complete flushings of the entire apheresis tubing system with LRS chambers.

## ACKNOWLEDGMENT

Open access funding enabled and organized by Projekt DEAL.

## CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

## ORCID

Viola Hähnel  <https://orcid.org/0000-0003-4115-8405>

Norbert Ahrens  <https://orcid.org/0000-0002-4680-2688>

## REFERENCES

1. Strauss RG. Risks of clinically significant thrombocytopenia and/or lymphocytopenia in donors after multiple plateletpheresis collections. *Transfusion*. 2008;48:1274–8.
2. Commission directive 2004/33/EC of 22 March 2004 implementing Directive 2002/98/EC of the European Parliament and of the Council as regards certain technical requirements for blood components L 91/25, 2004.
3. Boudreau G, Carli C, Lamarche C, Rulleau C, Bonnaure G, Neron S, et al. Leukoreduction system chambers are a reliable cellular source for the manufacturing of T-cell therapeutics. *Transfusion*. 2019;59:1300–11.
4. Néron S, Thibault L, Dussault N, Côté G, Ducas É, Pineault N, et al. Characterization of mononuclear cells remaining in the leukoreduction system chambers of apheresis instruments after routine platelet collection: a new source of viable human blood cells. *Transfusion*. 2007;47:1042–9.
5. Zidar DA, Al-Kindi SG, Liu Y, Krieger NI, Perzynski AT, Osnard M, et al. Association of Lymphopenia with Risk of mortality among adults in the US general population. *JAMA Netw Open*. 2019;2:e1916526.
6. Kishimoto S, Tomino S, Inomata K, Kotegawa S, Saito T, Kuroki M, et al. Age-related changes in the subsets and functions of human T lymphocytes. *J Immunol*. 1978;121:1773–80.
7. Warny M, Helby J, Nordestgaard BG, Birgens H, Bojesen SE. Lymphopenia and risk of infection and infection-related death in 98,344 individuals from a prospective Danish population-based study. *PLoS Med*. 2018;15:e1002685.
8. Zhao J, Gabriel E, Norda R, Høglund P, Baden L, Diedrich BA, et al. Frequent platelet donation is associated with lymphopenia and risk of infections: a nationwide cohort study. *Transfusion*. 2021;61:464–73.
9. Koepke JA, Parks WM, Goeken JA, Klee GG, Strauss RG. The safety of weekly plateletpheresis: effect on the donors' lymphocyte population. *Transfusion*. 1981;21:59–63.
10. Richa E, Krueger P, Burgstaler EA, Bryant SC, Winters JL. The effect of double- and triple-apheresis platelet product donation on apheresis donor platelet and white blood cell counts. *Transfusion*. 2008;48:1325–32.
11. Gansner JM, Rahmani M, Jonsson AH, Fortin BM, Brimah I, Ellis M, et al. Plateletpheresis-associated lymphopenia in frequent platelet donors. *Blood*. 2019;133:605–14.
12. Brosig A, Hähnel V, Orsó E, Wolff D, Holler E, Ahrens N. Technical comparison of four different extracorporeal photopheresis systems. *Transfusion*. 2016;56:2510–9.
13. Dullinger K, Pamler I, Brosig A, Mohrez M, Hähnel V, Offner R, et al. Granulocytapheresis with modified fluid gelatin versus high-molecular-weight hydroxyethyl starch: a matched-pair analysis. *Transfusion*. 2017;57:397–403.
14. Ahrens N, Thuer L. Data analysis script. *Protocols.io*. 2021. <https://doi.org/10.17504/protocols.io.bvv7n69n>.
15. Senhauser DA, Westphal RG, Bohman JE, Neff JC. Immune system changes in cytopheresis donors. *Transfusion*. 1982;22:302–4.
16. Prior CR, Coghlan PJ, Hall JM, Jacobs P. In vitro study of immunologic changes in long-term cytopheresis donors. *J Clin Apher*. 1991;6:69–76.

**FIGURE 6** Analysis of upper respiratory tract infections (URTI) in recent platelet donors with LRS apheresis: (A) percentage of days with URTI during the study period (URTI days) as a function of median lymphocyte count and (B) median annual platelet donation frequency. (C) Number of URTI during the study period (URTI count) as a function of median lymphocyte count and (D) median annual platelet donation frequency. (E) URTI duration between donations (if applicable) as a function of current lymphocyte count in all URTIs and (F) current annual platelet donation frequency in all URTIs. Colors from green to red (A)–(D) represent the number of study questionnaire participation days [Color figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

17. Strauss RG. Effects on donors of repeated leukocyte losses during plateletpheresis. *J Clin Apher.* 1994;9:130–4.
18. Robbins G, Petersen CV, Brozovic B. Lymphocytopenia in donors undergoing regular platelet apheresis with cell separators. *Clin Lab Haematol.* 1985;7:225–30.
19. Heal JM, Horan PK, Schmitt TC, Bailey G, Nusbacher J. Long-term follow-up of donors cytopheresed more than 50 times. *Vox Sang.* 1983;45:14–24.
20. Strauss RG, Huestis DW, Wright DG, Hester JP. Cellular depletion by apheresis. *J Clin Apher.* 1983;1:158–65.
21. Strauss RG. Apheresis donor safety-changes in humoral and cellular immunity. *J Clin Apher.* 1984;2:68–80.
22. Matsui Y, Martin-Alosco S, Doenges E, Christenson L, Shapiro HM, Yunis EJ, et al. Effects of frequent and sustained plateletpheresis on peripheral blood mononuclear cell populations and lymphocyte functions of normal volunteer donors. *Transfusion.* 1986;26:446–52.
23. Chang CJ, Chen LY, Liu LK, Lin MH, Peng LN, Chen LK. Lymphopenia and poor performance status as major predictors for infections among residents in long-term care facilities (LTCFs): a prospective cohort study. *Arch Gerontol Geriatr.* 2014;58:440–5.
24. Bender BS, Nagel JE, Adler WH, Andres R. Absolute peripheral blood lymphocyte count and subsequent mortality of elderly men. The Baltimore longitudinal study of aging. *J Am Geriatr Soc.* 1986;34:649–54.
25. Izaks GJ, Remarque EJ, Becker SV, Westendorp RG. Lymphocyte count and mortality risk in older persons. The Leiden 85-plus study. *J Am Geriatr Soc.* 2003;51:1461–5.
26. Rubio-Rivas M, Formiga F, Grillo S, Gili F, Cabrera C, Corbella X. Lymphopenia as prognostic factor for mortality and hospital length of stay for elderly hospitalized patients. *Aging Clin Exp Res.* 2016;28:721–7.
27. Gansner JM, Papari M, Goldstein J, Gaufer RA, Neuberger D, Makar RS, et al. Severe CD4+ T-cell lymphopenia is not observed in frequent plateletpheresis donors collected on the Fenwal amicus. *Transfusion.* 2019;59:2783–7.
28. Rahmani M, Fortin BM, Berliner N, Issa N, Neuberger D, Kaufman RM, et al. CD4+ T-cell lymphopenia in frequent platelet donors who have ceased platelet donation for at least 1 year. *Transfusion.* 2019;59:1644–7.
29. Moog R, Müller N. White cell reduction during plateletpheresis: a comparison of three blood cell separators. *Transfusion.* 1999;39:572–7.
30. Strasser EF, Weidinger T, Zimmermann R, Ringwald J, Eckstein R. Recovery of white blood cells and platelets from leukoreduction system chambers of Trima Accel and COBE spectra plateletpheresis devices. *Transfusion.* 2007;47:1943–4. author reply 4–5.
31. Lee Y, Kim S, Lee ST, Kim HS, Baek EJ, Kim HJ, et al. Analysis of characteristics of mononuclear cells remaining in the leukoreduction system chamber of Trima Accel and their differentiation into dendritic cells. *Korean J Lab Med.* 2009;29:353–60.
32. Gorlin JB, Commentary on Zhao et al. Frequent platelet donations is associated with lymphopenia, and risk of infections: A nationwide cohort study. *Transfusion.* 2021;61:1329–32.
33. Trepel F. Number and distribution of lymphocytes in man. A critical analysis. *Klin Wochenschr.* 1974;52:511–5.
34. Moir S, Donoghue ET, Pickeral OK, Malaspina A, Planta MA, Chun T-W, et al. Continuous flow leukapheresis induces expression of stress genes in lymphocytes: impact on microarray analyses. *Blood.* 2003;102:3852–3.
35. Karadoğan İ, Ündar L. Automated plateletpheresis does not cause an increase in platelet activation in volunteer donors. *Ther Apher.* 1997;1:174–7.
36. Stohlawetz P, Kapiotis S, Seidl D, Hergovich N, Zellner M, Eichler HG, et al. Safety issues of plateletpheresis: comparison of the effects of two cell separators on the activation of coagulation, fibrinolysis, and neutrophils and on the formation of neutrophil-platelet aggregates. *Transfusion.* 1999;39:420–7.
37. Liu X, Yuan L, Li D, Tang Z, Wang Y, Chen G, et al. Blood compatible materials: state of the art. *J Mater Chem B.* 2014;2:5718–38.
38. Weber M, Steinle H, Golombek S, Hann L, Schlensak C, Wendel HP, et al. Blood-contacting biomaterials: in vitro evaluation of the Hemocompatibility. *Front Bioeng Biotechnol.* 2018;6:99.
39. Huang Z, Gao D, Letteri JJ, Clark WR. Blood-membrane interactions during dialysis. *Semin Dial.* 2009;22:623–8.
40. Ghio M, Contini P, Ansaldi F, Ubezio G, Setti M, Risso M, et al. A possible role of soluble HLA-I molecule in the immunomodulatory effects of therapeutic apheresis. *Blood Transfus.* 2014;12(Suppl 1):s167–9.
41. Ghio M, Contini P, Ansaldi F, Ubezio G, Setti M, Risso M, et al. Immunomodulation due to plasma or plasma-platelet apheresis donation: events occurring during donation procedures. *J Clin Apher.* 2015;30:204–11.
42. Puppo F, Contini P, Ghio M, Brenci S, Scudeletti M, Filaci G, et al. Soluble human MHC class I molecules induce soluble Fas ligand secretion and trigger apoptosis in activated CD8(+) Fas (CD95)(+) T lymphocytes. *Int Immunol.* 2000;12:195–203.
43. Zimmermann R, Loew D, Weisbach V, Strasser E, Ringwald J, Zingsem J, et al. Plateletpheresis does not cause long-standing platelet-derived growth factor release into the donor blood. *Transfusion.* 2005;45:414–9.
44. Ghio M, Contini P, Ubezio G, Ansaldi F, Setti M, Tripodi G. Transient transforming growth factor beta1 modulation in monocytes and natural killer cells following plasma or plasma-platelet apheresis donation procedures. *Blood Transfus.* 2015;13:684–6.

## SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

**How to cite this article:** Thuer L, Brosig A, Hutchinson JA, Hähnel V, Offner R, Burkhardt R, et al. Total platelet donation count and donation frequency are determinants of plateletpheresis-associated lymphopenia. *Transfusion.* 2021;1–13. <https://doi.org/10.1111/trf.16666>