Changes in hematological indices and lymphocyte subsets in response to whole blood donation in healthy male donors

Anwar Borai, Callum Livingstone, Enaam Alsobhi, Abeer Al sofyani, Dalal Balgoon, Anwar Farzal, Mohammed Almohammadi, Abdulafattah Al-Amri, Suhad Bahijri, Daad Alrowaili, Wafaa Bassiuni, Ayman Saleh, Norah Alrowaili & Mohamed Abdelaal

To cite this article: Anwar Borai, Callum Livingstone, Enaam Alsobhi, Abeer Al sofyani, Dalal Balgoon, Anwar Farzal, Mohammed Almohammadi, Abdulafattah Al-Amri, Suhad Bahijri, Daad Alrowaili, Wafaa Bassiuni, Ayman Saleh, Norah Alrowaili & Mohamed Abdelaal (2017) Changes in hematological indices and lymphocyte subsets in response to whole blood donation in healthy male donors, Scandinavian Journal of Clinical and Laboratory Investigation, 77:2, 143-148, DOI: [10.1080/00365513.2017.1286520](https://doi.org/10.1080/00365513.2017.1286520)

To link to this article: <http://dx.doi.org/10.1080/00365513.2017.1286520>

Published online: 20 Feb 2017.

Submit your article to this journal [↗](#)

Article views: 28

View related articles [↗](#)

View Crossmark data [↗](#)

ORIGINAL ARTICLE

Changes in hematological indices and lymphocyte subsets in response to whole blood donation in healthy male donors

Anwar Borai^a, Callum Livingstone^b, Enaam Alsobhi^a, Abeer Al sofyani^a, Dalal Balgoon^a, Anwar Farzal^a, Mohammed Almohammadi^a, Abdulafattah Al-Amri^a, Suhad Bahijri^c, Daad Alrowaili^a, Wafaa Bassiuni^a, Ayman Saleh^a, Norah Alrowaili^a and Mohamed Abdelaal^a

^aKing Abdullah International Medical Research Center, King Saud bin Abdulaziz University for Health Sciences, Pathology, King Abdulaziz Medical City, Jeddah, Saudi Arabia; ^bFaculty of Health & Medical Sciences, University of Surrey, Guildford, Surrey, UK; ^cDepartment of Clinical Biochemistry, Faculty of Medicine, King Abdulaziz University, Jeddah, Saudi Arabia

ABSTRACT

Whole blood donation has immunomodulatory effects, and most of these have been observed at short intervals following blood donation. This study aimed to investigate the impact of whole blood donation on lymphocyte subsets over a typical inter-donation interval. Healthy male subjects were recruited to study changes in complete blood count (CBC) ($n = 42$) and lymphocyte subsets ($n = 16$) before and at four intervals up to 106 days following blood donation. Repeated measures ANOVA were used to compare quantitative variables between different visits. Following blood donation, changes in CBC and erythropoietin were as expected. The neutrophil count increased by 11.3% at 8 days ($p < .001$). Novel changes were observed in lymphocyte subsets as the CD4/CD8 ratio increased by 9.2% ($p < .05$) at 8 days and 13.7% ($p < .05$) at 22 days. CD16-56 cells decreased by 16.2% ($p < .05$) at 8 days. All the subsets had returned to baseline by 106 days. Regression analysis showed that the changes in CD16-56 cells and CD4/CD8 ratio were not significant (Wilk's lambda = 0.15 and 0.94, respectively) when adjusted for BMI. In conclusion, following whole blood donation, there are transient changes in lymphocyte subsets. The effect of BMI on lymphocyte subsets and the effect of this immunomodulation on the immune response merit further investigation.

ARTICLE HISTORY

Received 16 November 2016
Revised 6 January 2017
Accepted 21 January 2017

KEYWORDS

Blood transfusion and donation; B-lymphocyte subsets; flow cytometry; T-lymphocyte subsets; immunomodulation

Introduction

Blood transfusion is an important medical treatment. Because of the high demand for donated blood, researchers have sought to determine the minimum safe interval between donations. Currently, recommended minimum intervals range from 8–16 weeks in different countries, [1]. Researchers have examined the effects of blood donation on hematological parameters, aiming to assess both the acute impact and recovery time. Recovery of the blood hemoglobin (Hb) concentration and recovery of iron stores are the limiting factors for inter-donation intervals. For example, one study of 105 subjects showed that, following whole blood donation, the blood Hb concentration recovered after a mean interval of 49.6 days (range 18–98 days) [2]. A more recent study reported that, following whole blood donation (550 mL), red blood cell (RBC) mass recovered after a mean interval of 36 days (range 20–59 days) [3]. Hb, RBC and mean cell volume (MCV) have been reported to be lower in regular blood donors than in non-donors [4]. Recently, Baart et al. reported from a large study of donors, that a minimum donation interval of 56 days was sufficient only for donors in whom the blood Hb concentration was well above the cut-off level for donation [5]. These observations raise questions about whether the recommended intervals

between blood donations are appropriate for all regular donors.

Recent studies have observed effects of blood donation on components of the immune system, collectively known as immunomodulation. The activities of the humoral immune system are mediated by distinct classes or subsets of lymphocytes namely T-lymphocytes, B-lymphocytes and natural killer (NK) cells, distinguished by cell surface markers. The most abundant lymphocyte subsets are CD3 (Pan-T), CD3/4 (T-helper), CD3/8 (T-cytotoxic/suppressor), CD16-56 (natural killer (NK)) and CD19 (Pan-B). Changes in lymphocyte subsets have been observed early following blood donation. For example, human stem cells (HSCs), the principal marker of which is CD34, increased significantly at 6 h following blood donation and returned to baseline at 24 h [6]. CD16-56 (NK) cells have been observed to decrease following blood donation [7]. Recent evidence suggests that lymphocyte subset composition is influenced by body mass [8]. This raises the question of whether body mass influences the effect of blood donation on lymphocyte subsets.

A second reason for studying changes following blood donation is in order to understand the effects of hemorrhage, such as occur in patients who have suffered trauma, disease or who have undergone surgery. The effects of hemorrhage have been studied directly in such patients.

However, the volumes of blood lost vary widely. In addition, many factors other than blood loss are likely to be present which may deplete iron stores or affect functioning of the immune system. Such factors include the underlying disease, the effects of the acute phase response (APR) accompanying infection or illness and the effects of intravenous fluid and nutritional supplements given to patients. The study of healthy donors enables the effects of hemorrhage to be investigated in the absence of these confounding factors and enables the volume of blood lost to be standardized.

The immunomodulatory effects of blood loss may have clinical implications such as predisposing to infection or recurrence of cancer. Clinical outcomes following blood loss therefore merit investigation but their study is complicated in that patients who have suffered blood loss often undergo surgery which is itself immunomodulatory and likely to be immunosuppressive [9]. In addition, patients requiring surgery are a highly heterogeneous group with various other factors present which may affect functioning of the immune system. Studying the effects of blood donation of healthy subjects can help to establish which effects are attributable to blood loss rather than to other factors including a subsequent transfusion. Studies published to date which have examined immunomodulation following blood donation have followed patients over periods of a few weeks or less. In this study, we aimed to assess the effect of a single blood donation on lymphocyte subsets, extending the investigation to 106 days which is a typical inter-donation interval. Whilst the effects of blood donation on standard hematological parameters have previously been well described [10–12], we considered it important to investigate these in parallel with lymphocyte subsets, in order to confirm that the subjects' responses were typical.

Subjects and methods

Subjects

In total, 42 healthy male subjects were recruited to the study. Exclusion criteria were current acute illness, cancer, hematological disease, previous acute myocardial infarction, symptoms of cardiovascular disease, malabsorption, intestinal obstruction or any other condition contraindicating blood donation. Subjects were also excluded if they were being treated with prescribed medications or nutritional supplements such as iron or if fewer than 6 months had elapsed since a previous blood donation. All subjects gave written informed consent for participation. The study was approved by King Abdullah International Medical Research Center (KAIMRC) Ethical Committee (Study number RCJ0611-183).

Study protocol

Each participant attended the research center at 09:00 h on five occasions (Figure 1). At the first attendance (visit A), suitability for participation was assessed and body mass index (BMI) and blood pressure (BP) measured. A blood specimen (10 mL) was collected, by standard venesection

Figure 1. Study protocol for sampling from male donors before and after whole blood donation. In total, 42 subjects had CBC measurements and 16 had FC and EPO measured. Ten subjects attended visit E.

technique, into potassium ethylenediaminetetra-acetic acid (K-EDTA) for measurement of complete blood count (CBC), screening for abnormal Hb variants and analysis by flow cytometry (FC). A second specimen (5 mL) was collected into a plain tube for measurement of the serum erythropoietin (EPO) concentration. On the afternoon of the same day, whole blood donation (450 mL) was carried out by standard technique at the donation center of the blood bank. All donated blood was screened for infectious disease markers and was eligible for transfusion under CAP and AABB regulations. At 24 h following blood donation (visit B), specimens were taken for CBC, FC and EPO as described above. The same set of specimens was taken from all subjects who attended the subsequent visits at eight days (visit C), 22 days (visit D) and 106 days (visit E) following blood donation. Subjects were instructed to maintain their usual lifestyle and dietary habits throughout the study period and to avoid taking iron or other nutritional supplements.

Assays

CBC analyses were carried out on an Advia 2120i analyzer (Siemens, Munich, Germany). Hb was measured spectrophotometrically at 565 nm using a cyanide-free method. RBC indices namely mean cell volume (MCV), mean cell Hb (MCH), mean cell Hb concentration (MCHC) and hematocrit (Hct) were calculated by the analyzer.

FC measurements were performed on an FACSCantoTM II flow cytometer which incorporated Cell QuestTM software for data analysis (BD Biosciences, San Jose, CA, USA). This instrument uses a reagent containing fluorochrome-labelled antibodies which bind specifically to leukocyte surface antigens, staining the cells. RBCs were then lysed and the stained cells measured. Labelling of cells for direct immunofluorescence was performed using monoclonal antibodies (TBNK reagent, BD Biosciences, San Jose, CA). Cells

Table 1. Complete blood count (mean $\pm$ SEM) in healthy subjects pre- and 24 h, 8 days, 22 days and 106 days' post-donation.

	Unit	Pre-donation, <i>n</i> = 42	24 h, <i>n</i> = 42	Diff %	8 days, <i>n</i> = 42	Diff %	22 days, <i>n</i> = 42	Diff %	106 days, <i>n</i> = 10	Diff %	Repeated ANOVA	
											Sig	Eta
WBC	$\times 10^9/L$	6.20 $\pm$ 0.18	6.27 $\pm$ 0.21	1.2	6.47 $\pm$ 0.25	4.3	6.19 $\pm$ 0.24	-0.2	6.93 $\pm$ 0.65	11.9	0.200	0.039
RBC	$\times 10^{12}/L$	5.50 $\pm$ 0.08	5.09 $\pm$ 0.08***	-7.5	5.01 $\pm$ 0.08***	-9.0	5.11 $\pm$ 0.08***	-7.1	5.580 $\pm$ 0.24	1.4	<0.0001	0.642
Hb	g/L	159.8 $\pm$ 1.7	147.4 $\pm$ 1.6***	-7.7	144.9 $\pm$ 1.7***	-9.3	149.2 $\pm$ 1.7***	-6.6	154.9 $\pm$ 4.1	-3.0	<0.0001	0.622
Hct	%	48.36 $\pm$ 0.48	44.58 $\pm$ 0.46***	-7.8	44.19 $\pm$ 0.46***	-8.6	44.99 $\pm$ 0.46***	-7.0	46.70 $\pm$ 1.06***	-3.4	<0.0001	0.584
MCV	fL	86.79 $\pm$ 1.63	87.94 $\pm$ 0.86	1.3	88.52 $\pm$ 0.88	2.0	87.66 $\pm$ 1.12	1.0	84.36 $\pm$ 2.04*	-2.3	0.460	0.022
MCH	Pg	29.12 $\pm$ 0.35	29.11 $\pm$ 0.33	0.0	29.06 $\pm$ 0.34	-0.2	29.32 $\pm$ 0.03*	0.7	28.01 $\pm$ 0.87	-3.8	0.016	0.084
MCHC	g/L	329.9 $\pm$ 1.8	330.3 $\pm$ 2.0	0.1	328.1 $\pm$ 1.7	-0.5	331.6 $\pm$ 1.7	0.5	331.5 $\pm$ 3.6	0.5	0.253	0.034
RDW	%	13.03 $\pm$ 0.10	13.01 $\pm$ 0.11	-0.1	13.38 $\pm$ 0.13***	2.6	13.39 $\pm$ 0.13***	2.7	13.36 $\pm$ 0.24	2.5	<0.0001	0.473
PLT	$\times 10^9/L$	263 $\pm$ 8	261 $\pm$ 8	-0.8	285 $\pm$ 7***	8.7	276 $\pm$ 8*	5.0	277 $\pm$ 23	5.4	<0.0001	0.242
MPV	fL	7.05 $\pm$ 0.17	7.27 $\pm$ 0.17	3.1	7.00 $\pm$ 0.16	-0.8	7.45 $\pm$ 0.18	5.6	7.15 $\pm$ 0.33	1.4	0.153	0.044
NEU	%	50.20 $\pm$ 2.04	52.22 $\pm$ 1.74	4.0	55.84 $\pm$ 1.28**	11.3	54.13 $\pm$ 1.49	7.8	59.05 $\pm$ 3.84	17.6	0.011	0.09
LYM	%	32.72 $\pm$ 1.62	32.81 $\pm$ 1.43	0.3	31.57 $\pm$ 1.18	-3.5	32.84 $\pm$ 1.32	0.4	29.02 $\pm$ 3.08	-11.3	0.594	0.016

The percentage differences (Diff%) values shown refer to the difference between each variable at the given time compared to pre-donation. * $p < .05$, ** $p < .01$, *** $p < .001$ compared to pre-donation. Ten subjects attended at 106 days. Differences at 106 days are based on the results of the same subjects' pre-donation. Repeated ANOVA indices were calculated based the first four visits. Eta, partial Eta squared, i.e. the percentage of variance that is accounted for by the effect of blood donation; Hb: hemoglobin; Hct: hematocrit; LYM: lymphocytes; MCV: mean cell volume; MCH: mean cell hemoglobin; MCHC: mean cell hemoglobin concentration; MPV: mean platelet volume; *n*: number of subjects; NEU: neutrophils; PLT: platelet count; RBC: red blood cells; RDW: red cell distribution width; Sig: the estimate of within-subject mean variation across different time-points using repeated ANOVA; WBC: white blood cells.

were detected using the antigenic CD3, CD4, CD8, CD19 and CD16-56 cell surface markers.

Serum EPO concentrations were measured by chemiluminescent immunoassay on an Immulite 1000 analyzer (Siemens, Munich, Germany). Screening for abnormal Hb variants was by high pressure liquid chromatography (HPLC) using a TOSOH Bioscience, Inc instrument (South San Francisco, CA, USA). All analyses were carried out in a laboratory fully accredited by the College of American Pathologists (CAP). All mandatory laboratory health and safety procedures were complied with during experimental work.

Statistics

Before statistical analysis was carried out, the distribution of data and homogeneity of variances were evaluated using Levene's test. Data were log transformed if necessary. Comparisons of baseline data and data obtained following blood donation were carried out by paired *t*-test. Repeated measures ANOVA was performed as a separate analysis when necessary. Eta squared was used in the ANOVA model to measure the effect size of blood donation on each variable. In all cases, $p < .05$ was taken as the level of statistical significance. Results are given as mean $\pm$ SEM unless otherwise stated.

Results

Subject characteristics

The 42 male subjects were aged 36 ± 1.1 years (mean $\pm$ SEM) (range 23–54 years) and had BMI 27.3 ± 0.5 kg/m² (range 20.8–35.2 kg/m²). None of the subjects experienced symptoms or acute adverse effects, such as significant decrease in BP, following blood donation. None of the subjects had abnormal Hb variants. Only 16 of the subjects had FC and

EPO measurements carried out and only 10 of the subjects who started the study attended for the final visit at 106 days. In total, there were 178 subject visits.

Hematological parameters and erythropoietin

Data for CBC and white blood cell (WBC) categories are presented in Table 1. Hb concentration, RBC count and Hct were significantly lower than pre-donation values at 24 h, 8 days and 22 days following blood donation ($p < .001$). The three parameters decreased in similar trends, the decreases ranging from 6.6–9.3%. By 106 days these parameters, with the exception of Hct, had returned to levels not statistically significant from baseline. RBC distribution width (RDW) was significantly increased at 8 and 22 days' post-donation ($p < .001$). MCV and MCHC did not change in the first 22 days' post-donation but there was a slight decrease in MCV at 106 days ($p < .05$).

The total WBC count did not change significantly at any interval following blood donation. However, the differential WBC count showed that the neutrophil fraction increased by 11.3% at 8 days ($p < .001$). The lymphocyte fraction was decreased at 8 days but this was not statistically significant. No significant differences were observed in the other components of the differential count. Platelet counts were increased significantly at 8 ($p < .001$) and 22 days ($p < .05$) with a variance of 24.2% ($p < .0001$) but had returned to baseline at 106 days. Data on serum erythropoietin concentrations are shown in Table 2. After whole blood donation, EPO increased significantly ($p < .01$) at 24 h and at 8 and 22 days by 72.5, 45.3 and 42.9%, respectively. The variance of measurements across visits was 27% ($p < .019$).

Lymphocyte subsets

Data on lymphocyte subsets measured by FC are presented in Table 2. At 8 days after blood donation CD3 and CD4

Table 2. Serum erythropoietin concentrations and lymphocyte subsets by flow cytometry (mean $\pm$ SEM) in healthy subjects pre-and 24 h, 8 days, 22 days and 106 days' post-donation.

	Unit	Pre-donation, <i>n</i> = 16	24 h, <i>n</i> = 16	Diff %	8 days, <i>n</i> = 16	Diff %	22 days, <i>n</i> = 16	Diff %	106 days, <i>n</i> = 10	Diff %	Repeated ANOVA	
											Sig	Eta
EPO	IU/L	9.6 $\pm$ 1.7	16.6 $\pm$ 2.1**	72.5	14.0 $\pm$ 2.5**	45.3	13.8 $\pm$ 2.2**	42.9	12.1 $\pm$ 2.1	25.8	0.019	0.27
CD3	%	67.44 $\pm$ 2.31	67.81 $\pm$ 1.93	0.6	69.20 $\pm$ 2.27*	2.6	69.13 $\pm$ 2.01	2.5	68.3 $\pm$ 3.13	2.0	0.059	0.172
CD4	%	35.38 $\pm$ 1.71	35.56 $\pm$ 1.56	0.5	37.60 $\pm$ 1.74*	6.3	37.53 $\pm$ 1.78	6.1	34.9 $\pm$ 1.64	−0.5	0.167	0.12
CD8	%	29.19 $\pm$ 1.77	29.19 $\pm$ 1.66	0.0	28.13 $\pm$ 1.60	−3.6	26.60 $\pm$ 1.68	−8.9	27.7 $\pm$ 2.42	−2.9	0.166	0.121
CD19	%	12.50 $\pm$ 1.04	12.88 $\pm$ 1.46	3.0	13.87 $\pm$ 1.44	10.9	13.33 $\pm$ 1.34	6.7	12.7 $\pm$ 1.64	−0.8	0.906	0.014
CD16-56	%	19.56 $\pm$ 2.41	18.56 $\pm$ 2.01	−5.1	16.40 $\pm$ 2.53*	−16.2	17.07 $\pm$ 2.00	−12.8	18.4 $\pm$ 3.41	−7.6	0.120	0.137
CD4/CD8		1.29 $\pm$ 0.11	1.30 $\pm$ 0.11	1.1	1.40 $\pm$ 0.11*	9.2	1.46 $\pm$ 0.12*	13.7	1.3 $\pm$ 0.141	4.0	0.039	0.19

The percentage differences (Diff%) values shown refer to the difference between each variable at the given time compared to pre-donation. * $p < .05$ compared to pre-donation. Sixteen subjects had FC analysis carried out. Ten subjects attended at 106 days. Differences at 106 days are based on the results of the same subjects' pre-donation. Repeated ANOVA indices were calculated based on the first four visits. EPO: erythropoietin; Eta, partial Eta squared, i.e. the percentage of variance that is accounted for by the effect of blood donation; *n*, number of subjects; sig, the estimate of within-subject mean variation across different time-points using repeated ANOVA.

cells constituted a higher proportion of the total lymphocyte count than at baseline. There was a non-significant decrease in CD8 cells as a proportion of total lymphocytes which, together with the increased proportion of CD4 cells, resulted in a significant increase (9.2%, $p < .05$) in the CD4/CD8 ratio. The CD4/CD8 ratio remained increased at 22 days (13.7%, $p < .05$) returning to baseline by 106 days. At 8 days, the proportion of CD16-56 (NK) cells as a percentage of total lymphocytes was significantly decreased (16.2%, $p < .05$). All lymphocyte subsets and the CD4/CD8 ratio had returned to pre-donation levels at 106 days. The proportion of CD19 cells did not change significantly throughout the study period.

The data were re-examined with the subjects divided into low BMI (BMI ≤ 28.0 kg/m²; $n = 10$) and high BMI (> 28.0 kg/m²; $n = 6$). Pre-donation CD16-56 (NK) cells as a proportion of total lymphocytes were $20.40 \pm 3.77\%$ in subjects with low BMI and $18.17 \pm 1.78\%$ in subjects with high BMI. There were no significant differences at 8 or 22 days following blood donation in the proportion of CD16-56 (NK) cells in subjects with high BMI while in subjects with low BMI CD16-56 were significantly decreased at 8 ($15.20 \pm 3.52\%$, $p < .01$) and 22 ($16.00 \pm 2.73\%$, $p < .01$) days following blood donation. The pre-donation CD4/CD8 ratio was 1.29 ± 0.11 in subjects with low BMI and 1.28 ± 0.61 in subjects with high BMI. There were no significant differences at any point following blood donation in subjects with high BMI whereas in subjects with low BMI the CD4/CD8 ratio was significantly increased at 22 days (1.45 ± 0.46 , $p < .05$). Regression analysis using ANOVA showed no significant changes in both CD16-56 cells and CD4/CD8 ratio when adjusted for BMI (Wilk's lambda = 0.15 and 0.94, respectively).

Discussion

In this study, we followed changes in lymphocyte subsets, concurrently with changes in CBC over 106 days, a longer period than in previous studies. In line with the findings of other studies [10–12] Hb and RBC count decreased significantly by 24 h following donation, remaining so until 22 days' post-donation, but there were no changes in MCV or

MCHC. The increased RDW at 8 days in the presence of normal MCV is a characteristic finding following recent blood loss, reflecting an increased range of RBC size. The increase in serum EPO concentration was similar to that previously observed by others [13]. These findings are to be expected, resulting from physiologic changes occurring after blood loss. There is an immediate redistribution of fluid from the extravascular to intravascular space which restores blood volume but results in a decreased RBC mass. This leads to an increase in the serum EPO concentration, stimulating RBC production, and eventually restoring RBC mass [14]. The increase in platelet count which we observed may be explained by an influx of young and sequestered platelets into the circulation in response to blood loss. Whilst these hematological changes have previously been described elsewhere [10–12], it was reassuring to confirm that the responses were as expected before considering immunomodulatory effects.

Although blood donation had no effect on the total WBC or lymphocyte counts, it had a significant effect upon the differential WBC count showing an increased fraction of neutrophils at 8 days. Similar changes have been reported in previous studies. A study by Karger et al. [7] which investigated post-blood donation changes in CBC reported that, at about 2 weeks following blood donation, the total leukocyte count did not change but the granulocyte count, the majority of which are neutrophils, had increased significantly by 4.6% and the lymphocyte count decreased significantly by 10.5%. The higher percentage increase in neutrophils (11.3%) in our study may reflect the earlier time-point of sampling following the acute insult of blood donation. By carrying out a longer study than Karger et al., we demonstrated that, by 106 days' post-donation, the neutrophil count had returned to normal. Neutrophils secrete pro-inflammatory cytokines which are a vital component of the APR [15]. Possible causes of neutrophil leukocytosis therefore include infection or inflammation but these are unlikely in this group of healthy donors. A more likely reason is increased secretion of the stress hormones cortisol and epinephrine [16]. These hormones are secreted in response to blood loss resulting in mobilization of leukocytes from bone marrow and from the marginalized pool of neutrophils

adherent to blood vessel walls. When neutrophil leukocytosis is persistent, it is associated with autoimmune disease, cardiovascular disease and cancer [17]. Post-donation neutrophil leukocytosis is transient and therefore unlikely to have adverse consequences for healthy donors.

Whilst blood donation had no effect on the total lymphocyte count, it had significant effects upon the lymphocyte subsets. We observed a decrease in CD16-56 (NK) cells, at all points following donation, which was statistically significant at 8 days (16.2%). Decreases in CD16-56 (NK) cells have been observed previously following blood donation in healthy donors [18]. Interestingly, these authors observed decreased NK cell activity only in subjects who had cumulatively donated moderate volumes of blood (3–9 L). No change was observed in subjects who had donated high volumes of blood (>9 L).

Decreases in CD16-56 (NK) cells have also been observed in patients who have undergone blood donation [7,19]. In patients awaiting elective hip surgery [7], there was a 20% decrease in CD16-56 (NK) counts in the second week post-donation, similar to our finding at 8 days post-donation. Decreased CD16-56 (NK) cell counts have also been observed following blood donation in patients with cancer [19]. These authors observed that, at 12 days' post-donation, CD16-56 (NK) cells were 54% of pre-donation levels. The implications of this finding are uncertain but NK cells are known to have an anti-tumor role in preventing tumor recurrence [20]. Decreased NK cell activity was associated with an increased incidence of cancer in an 11-year prospective study [21]. It could be anticipated that a decrease in NK cells might predispose to cancer recurrence. However, the short duration of the decrease in NK cells in studies suggests that it would be unlikely affect cancer recurrence. It is uncertain why the decrease in NK cells varies widely between studies but this could reflect the clinical characteristics of the study subjects. For example, it would be expected that the stimuli to NK cell production would be different in patients with cancer compared to those awaiting elective surgery or compared to healthy donors. A potentially more important consequence of hemorrhage-related immunomodulation in hospitalized patients is an increased risk of post-operative infection but no study has investigated this specifically.

There was no significant change in CD8 cells but CD3 and CD4 cells increased significantly at 8 days and there was a significant and sustained increase in the CD4/CD8 ratio. Karger et al. [7] also reported an increase in the CD4/CD8 ratio following blood donation. This increase may be explained, at least in part, by the concurrent increase in the serum EPO concentration. The effects of EPO on blood cell development were previously thought to be confined to erythroid cells, but recent studies have observed that various types of cells in the immune system are affected [14]. This suggests that EPO should be considered a pleiotropic cytokine. In animal models, it has been found to have anti-inflammatory effects and to inhibit monocyte function [22] by inhibiting the NF- κ B pathway [14]. The CD4/CD8 ratio has been linked to activation of the immune system [23]. Various lines of evidence suggest that a declining CD4/CD8

ratio correlates with immune dysfunction leading to a poor response to immunization, as well as increased risk of severe infections and malignancies [24,25]. It also predicts mortality in patients with viral infection and in the general population [26]. The significance of the increased CD4/CD8 ratio in response to blood donation is uncertain but it may reflect a transient immune activation due to blood donation, similar to the neutrophil leukocytosis. Although the mechanistic basis of the changes is uncertain, their observation in healthy donors, as opposed to hospitalized patients, indicates that they were attributable to blood donation rather than another factor such as a subsequent transfusion.

Although our study was not designed to investigate the effect of BMI on changes following blood donation we examined the available data on the subjects divided into those with low or high BMI. Both groups showed similar responses in the CBC parameters (data not shown). In subjects with high BMI there were no significant changes in the proportion of different lymphocytes subsets at any time-point following blood donation compared to baseline. However, in subjects with low BMI there was a significant decrease in CD16-56 (NK) cells at 8 and 22 days compared to baseline and an increase in the CD4/CD8 ratio at 22 days. The reason for these changes is unclear but it could in part reflect the practice of taking a standard volume of blood rather than adjusting the volume according to the BMI of the donor. The insult from blood loss would be expected to be proportionately higher in individuals with low BMI. Regression analysis of the data adjusted for BMI showed that, in the small number of subjects studied, the changes in lymphocyte subsets were not significant. The effect of BMI on lymphocyte subsets following blood donation merits further investigation by a suitably designed study on a larger number of subjects. In addition, future studies should investigate the effect of donors' blood volume measured using labelled RBCs.

Immune system functioning has previously been investigated in lean and obese subjects [8]. In line with our pre-donation observations, these authors reported that there were no differences in NK cell counts between lean and obese subjects, but they did not investigate the effects of blood donation. Recently NK cells from obese subjects have been observed to have functional deficits [27] which, over long-term periods could potentially contribute to the increased incidence of cancer in obesity [28]. It is unknown whether the transient decrease in CD16-56 (NK) cells observed in our study has adverse consequences for donors but it does suggest that subjects of different BMI may tolerate blood donation differently in terms of its effects on the immune system.

This study has a number of limitations, principally the relatively small number of subjects studied throughout and especially at 106 days and the small number of FC measurements. In addition, the study was confined to male subjects and was not specifically designed to investigate subjects of different BMI. It should be emphasized that whilst the changes observed in this study were statistically significant, they are not necessarily medically relevant.

In conclusion, this study observed significant changes in lymphocyte subsets following a single blood donation in male subjects. All changes immune system had stabilized by 106 days' post-donation. It is unclear whether the observed immunological changes have adverse or beneficial effects on regular donors but the effects and their consequences merit further investigation. In future studies, it would be desirable to measure more subtypes of CD16-56 (NK) cells and in a larger number of subjects. It would also be worthwhile to measure the concentrations of stress hormones and cytokines to investigate the mechanistic basis of changes in lymphocyte subsets. Such studies may inform recommendations on the minimum interval between donations.

Acknowledgements

The authors gratefully acknowledge funding support from the Saudi Government represented by the Saudi National Guard Health Affairs at King Abdullah International Medical Research Center – Western Region (KIMRC-WR).

Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

Funding

The funding support was from King Abdullah International Medical Research Center – Western Region (KAIMRC-WR), Saudi Arabia-Jeddah [Grant number RCJ0611-183, Aug 14th 2012].

References

- PAHO. Eligibility for blood donation: recommendations for education and selection of prospective blood donors. ISBN: 978-92-75-12939-5. 2009:43-4.
- Fowler WM, Barer AP. Plasma iron; normal values; response following medication. *Am J Med Sci* 1952;223:633-41.
- Pottgiesser T, Specker W, Umhau M, Dickhuth HH, Roecker K, Schumacher YO. Recovery of hemoglobin mass after blood donation. *Transfusion* 2008;48:1390-7.
- Dhanasree VS, Naidu RS, Naidu VD, Naidu SS, Neelagiri A, Meghanna R. Study of various physiological, biochemical and hematological parameters in repeated blood donors. *J Health Sci* 2013;1:57-9.
- Baart AM, van den Hurk K, de Kort WL. Minimum donation intervals should be reconsidered to decrease low hemoglobin deferral in whole blood donors: an observational study. *Transfusion* 2015;55:2641-4.
- Pala C, Mumcuoglu H, Kurnaz F, Sivgin S, Kynar L, Keklik M, Akyol G, Koker Y, Eser B. Changes in CD34+ cell count in peripheral blood after whole blood donation. *Transfus Apher Sci* 2013;49:259-62.
- Karger R, Weber C, Schmidt J, Kretschmer V. Characterization of immune system alterations following preoperative autologous blood donation for elective hip replacement surgery. *Transf Med* 2007;17:45-53.
- Nieman DC, Henson DA, Nehlsen-Cannarella SL, Ekkens M, Utter AC, Butterworth DE, Fagoaga OR. Influence of obesity on immune function. *J Am Diet Assoc* 1999;99:294-9.
- Angele MK, Faist E. Clinical review: immunodepression in the surgical patient and increased susceptibility to infection. *Crit Care* 2002;6:298-305.
- Lister J, McNeill IF, Marshall VC, Plzak LF Jr, Dagher FJ, Moore FD. Transcapillary refilling after hemorrhage in normal man: basal rates and volumes; effect of norepinephrine. *Ann Surg* 1963;158:698-712.
- Adamson J, Hillman RS. Blood volume and plasma protein replacement following acute blood loss in normal man. *J Am Med Assoc* 1968;205:609-12.
- Ebert RV, Sej GJ. Response of normal subjects to acute blood loss. *Arch Intern Med* 1941;68:578.
- Ziegler AK, Grand J, Stangerup I, Nielsen HJ, Dela F, Magnussen K, Helge JW. Time course for the recovery of physical performance, blood hemoglobin, and ferritin content after blood donation. *Transfusion* 2015;55:898-905.
- Broxmeyer HE. Erythropoietin surprises: an immune saga. *Immunity* 2011;34:6-7.
- Kasama T, Miwa Y, Isozaki T, Odai T, Adachi M, Kunkel SL. Neutrophil-derived cytokines: potential therapeutic targets in inflammation. *Curr Drug Targets Inflamm Allergy* 2005;4:273-9.
- Franchimont D. Overview of the actions of glucocorticoids on the immune response: a good model to characterize new pathways of immunosuppression for new treatment strategies. *Ann N Y Acad Sci* 2004;1024:124-37.
- Arya M, Patel HR, Williamson M. Chemokines: key players in cancer. *Curr Med Res Opin* 2003;19:557-64.
- Lasek W, Jakobisiak M, Grochowska M, Plodzisewska M, Szczytnicki W. Two patterns of NK activity changes following blood donation: decrease in the beginners and restoration in regular blood bank donors. *Arch Immunol Ther Exp* 1992;40:191-4.
- Marquet RL, Hoynck van Papendrecht MA, Busch OR, Jeekel J. Blood donation leads to a decrease in natural killer cell activity: a study in normal blood donors and cancer patients. *Transfusion* 1993;33:368-73.
- Roitt IMDJ. Roitt's essential immunology. 10th ed. Oxford: Blackwell Science; 2004.
- Imai K, Matsuyama S, Miyake S, Suga K, Nakachi K. Natural cytotoxic activity of peripheral-blood lymphocytes and cancer incidence: an 11-year follow-up study of a general population. *Lancet* 2000;356:1795-9.
- Nairz M, Sonnweber T, Schroll A, Theurl I, Weiss G. The pleiotropic effects of erythropoietin in infection and inflammation. *Microbes Infect* 2012;14:238-46.
- Sainz T, Serrano-Villar S, Diaz L, Gonzalez-Tome MI, Gurbindo MD, de Jose MI, Mellado MJ, Ramos JT, Zamora J, Moreno S, Munoz-Fernandez MA. The CD4/CD8 ratio as a marker T-cell activation, senescence and activation/exhaustion in treated HIV-infected children and young adults. *AIDS* 2013;27:1513-16.
- Strindhall J, Nilsson BO, Lofgren S, Ernerudh J, Pawelec G, Johansson B, Wikby A. No immune risk profile among individuals who reach 100 years of age: findings from the Swedish NONA immune longitudinal study. *Exp Gerontol* 2007;42:753-61.
- Wikby A, Maxson P, Olsson J, Johansson B, Ferguson FG. Changes in CD8 and CD4 lymphocyte subsets, T cell proliferation responses and non-survival in the very old: the Swedish longitudinal OCTO-immune study. *Mech Age Dev* 1998;102:187-98.
- Lu W, Mehraj V, Vyboh K, Cao W, Li T, Routy JP. CD4:CD8 ratio as a frontier marker for clinical outcome, immune dysfunction and viral reservoir size in virologically suppressed HIV-positive patients. *J Int AIDS Soc* 2015;18:20052.
- Laue T, Wrann CD, Hoffmann-Castendiek B, Pietsch D, Hubner L, Kielstein H. Altered NK cell function in obese healthy humans. *BMC Obes* 2015;2:1.
- Renehan AG, Tyson M, Egger M, Heller RF, Zwahlen M. Body-mass index and incidence of cancer: a systematic review and meta-analysis of prospective observational studies. *Lancet* 2008;371:569-78.