

INFLUENCE OF THE TYPE OF PLATELETPHERESIS ON THE VALUE OF CORPUSCULAR ELEMENTS IN THE BLOOD DONORS

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SUMMARY

Introduction: During the plateletpheresis procedure the number of trombocytes in the donor's blood significantly decreases, and the levels of the other components of blood as hematocrit, hemoglobin, and leukocyte diminish as well. Influence of the type of procedure DN-CFCS and SN-ICFS it is one of the factors that affects the decrease of the levels of HCT, Hgb and WBC. In this study, our goal was to see the difference in the value of HCT, Hgb, WBC, and platelets after the plateletpheresis process between DN-CFCS and SN-IFCS on the same cell separator - Fenval AMICUS.

Donors and methods: The criteria for participation: men between age of 25-45. Two groups were formed. Group I 112 separation done with the method SN-ICFS and Group II 180 separation done with the method DN-CFCS. Statistical analysis: To confirm the statistical difference we used Student t-test for independent or dependent samples, as well as Mann-Whitney U test as non parametric alternative. The possibility of errors were accepted for $\alpha < 0.05$, and the difference between groups were accepted as statistical relevant for $p < 0.05$.

Results Statistically significant lower values were observed of all researched parameters after separation for the donors on the equipment Amicus DN, and for donors on Amicus SN. A significant higher value of HCT before procedure was found in the AM DN group, in the researches of the other variables there were no significant differences. The result for the comparison of variables after procedure for DN and SN procedure. A significant higher value of HCT and a significant higher level of Hgb, as well as a significant lower level of WBV after procedure in the AM DN group, while for the levels of PLT there were no significant differences.

Conclusion: On the decrease of the value of the observed parameters the type of procedure has an influence that means DN-CFCS or SN-IFCS, continuous or discontinuous flow.

Key words: Comparison of single needle versus double needle platelet, apheresis, donor hematological value

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INTRODUCTION

Collecting platelets with apheresis is considered as one of the biggest progress in transfusion medicine. It permits an adequate response to the fast increasing need for blood components. New technologies have permitted a more often donation of platelets than whole blood, a decrease in the number of leukocytes without filter, and the donors are filled with the feeling that they have in a particular way helped someone, that is saved a life.

To administrate such platelets have significantly decreased the risk for alloimmunization. Apheresis (from the Greek word *φαίρεσις* – apheresis, separate) is a term that is used to describe several forms of extracorporeal separation of blood. It is a medical procedure of separating blood components, to extract certain components and again returning the blood into the circulation.

The procedure is used in the treatment of certain diseases and when collecting plasma, platelets, cell components and similar.

The exact name of this procedure is platelet pheresis, but it is more correct to use the name single donor platelets apheresis. This procedure has a greater amount

of platelets (absolute number, yield and potential) that is present in doses of platelets than that obtained from single doses.

Roughly 5-15 single doses, obtained from whole blood, are equal to one dose obtained by apheresis. During the method called apheresis, the donor's blood passes through the cell separator, where with the method of centrifugation the blood is separated according to specific weight in several layers and that is a layer of eritocytes, leukocytes, platelets and plasma.

In parallel to the increase in the demand of platelet concentrations the techniques for obtaining them have improved: Platelet concentrates for transfusion (AABB Technical Manual, 17th edition, 2013) can be obtained in several ways and that is: from donation of whole blood with anticoagulants (plasma rich in platelets (PRP) and with the buffy coat method and apheresis on different types of equipments).

The purpose of the plateletpheresis procedure is obtaining a concentrate of trombocytes for the needs of curing patients. During this procedure the number of trombocytes in the donor's blood significantly decreases, and the levels of the other components of blood as Hct, Hgb and WBC diminish as well. Several authors state the

influence also of the type of procedure DN-CFCS and SN-ICFS as one of the factors that affects the decrease of the levels of Hct, Hgb and WBC. Some procedures include two veinpunctions (double needle-DN) and continuous flow (CFCS), and the other veinpunction (single needle-SN) with alternation of return and taking blood from one vein (intermitent-flow - cell-separation IFCS). Most of todays equipments use both methods according to which program is choosen on the separator. The main differences between the two procedures are the following:(Guidance for Industry and FDA (2012) Review Staff: Collection of Platelets by Automated Methods; Guide to preparation, use and quality assurance of blood components, (2010): Wollersheim et al. 2006; 34:179-86 (Table 1).

Table1. Main differences between IFCS-SN and CFSC – DN

IFCS – SN	CFCS - DN
Blood is processed in specific amounts, „meals” that are tolerated by the organism	Blood is processed and separated in a continuous flow.
When a certain amount of blood is processed, that is separation of platelets, the separation chamber must be emptied before a new filling can begin again.	When the procedure starts the separation chamber does not empty until the procedure is completed.
Need of a bigger extracorpular volume (ECV)	Need of a small or medium extracorpular volume (ECV)

The undeniable fact is that the platelet count after the separation drastically drops because the procedure was performed to extract a certain amount of platelets. However, the values of other parameters such as hematocrit, hemoglobin, and leukocytes are reduced in varying percentages, as stated by numerous authors (Benjamin et al. 1999, Bueno et al. 2005, Edwin et al. 2004, Rajendra et al. 2009, Veihola et al. 2006, Fontana et al. 2006, Fontana et al. 2011, Hans-Gert et al. 2013, Al-Raha 2012). Donor health is protected so that we never process in one session more than one total blood volume (TBV) donors, and we make a sufficient time interval between procedures.

In this study, our goal was to see the difference in the value of HCT, Hgb, WBC, and platelets after the plateletphreze process between DN-CFCS and SN-IFCS on the same cell separator in this case Fenwal AMICUS.

DONORS AND METHODS

The results obtained during regular work at the Institute of Transfusion Medicine of F B&H, Sarajevo, Bosnia and Hercegovina (ZZTMFBiH). of platelet citopheresis were used in this study.

As sample we used all those donors on the list of ZZTMFBiH that had at least been three times blood donors and that had accepted to participate in this procedure. From the register of blood donors ZZTMFBiH using the method of random selection-lottery, the blood donors were choosen to be platelet donors from the list of donors, depending on the need for platelets of certain blood groups, the donor's ability to reach the necessary time, the distance from the Institute, the time of previous donation, the current state of health, etc. It means, that was used this random selection for the sample of respondents from the the list of donors that all were donors of platelets as well.

The donors fulfilled all the recommendations of the council of Europe as well as the domestic legislation. Before the procedure the details of the method and the eventual negative side effects during the procedure were explained to the donors. All the donors that participated in the platelet citopheresis procedure gave their written consent that the obtained results can be used in a scientific research and published with the protection of the donor's identity.

The criteria for participation in the study: fulfill standard transfusiological criterias, men between age of 25-45; men donors which have had several plateletpheresis using SN and DN procedures on Fenwal AMICUS, for the study were used the first three separations and donors with a number of platelet before procedure above $150 \times 10^9/L$ and less than $450 \times 10^9/L$; with expected number of platelets – Yield $\geq 2 \times 10^{11}/L$, with procedure not stopped or shortened; values of platelets(PLT), hematocrits (HCT), hemoglobine(Hgb) and leukocytes (WBC)of the donor after procedure, valuesof platelets, hematocrits, hemoglobine and leukocytes of the donor before procedure : values of hematocrites $> 0,40\%$, values of hemoglobine $\geq 12,5$ g/dL; values of leukocytes 4-10 $K/\mu L$; measurements of the total amount of blood processed, the duration of the separation, the amount of anticoagulant used, the volume of product and number of platelet obtained after procedure.

Criteria for exclusion of the study: donors for which the procedure was stopped or shortened. According to the established goal and conditions for entering the study as well as being excluded from the study two groups were formed.

Group I 112 separation is formed by 60 participants were the values of hematokrits, the concentration of hemoglobin and number of leukocytes are established before and after separtion done with the method SN-ICFS on the Fenwal AMICUS.

Group II 180 separation is formed by 60 participants were the values of hematokrits, the concentration of hemoglobin and number of leukocytes are established before and after separtion done with the method DN-CFCS on the Fenwal AMICUS.

Our donors are men under 45 years with body height (TV) between 1.68 m and 2.06 m and the arithmetic mean is 1.82 m and the standard deviation (STDev) is 0.07. The body mass (TM) of the donor is between 61 kg and 168 kg with a arithmetic mean of 93.2 kg. And STDev from 15.50. From these data, the total donor blood volume (TBV) ranges from 4 370.27 ml to 9219.39 ml with arithmetic mean of 5811.89 ml and STDev 698.36. The number of donors who completed all of the study conditions was 74 and we took the first 60 donors in the study who completed 180 DN procedures and 112 SN procedures.

For the study the following equipment were used: cell separator – Fenwal AMICUS, sets for single use FENWAL AMICUS KIT R4R 2314 and FENWAL AMICUS KIT R4R 2326, anticoagulants ACD 1L 4B7891; ACD 0.5 L 4B7898 and needles for hemapheresis Baxter RAM 5033 T 17G.

Methodology of taking and analysing sample

Parameters that can be changed but are given as defaults before procedure, are: extra volume of physiological solution that is returned to the donor at the end of the procedure 60mL, maximum blood flow is 70mL/min, limit of the input pressure - 250 mm Hg, limit of the return pressure – 450 mm Hg, pressure on the manometer on the donors arm above the venepunction 50mmHg, citrat rate 1.25mg/kg and ACD ratio 1:10.

The values of platelets, hematokrits, hemoglobin and leukocytes of the donor, before and after procedure, are established with Electronic counter CellDyne 3200 (Abbot Laboratories, IL, USA). Samples for these parameters were taken with eprouvettes (vacutainer with EDTA 5.4 mg 3 ml blood) from the vein that will not be used during the procedure. The sample of blood after procedure is taken from the entering vein after the end

of the procedure and after we have taken 5ccm³ blood in the eprouvette from which we will not do this measurement.

Statistical analysis

The datas obtained (HCT, WBC, PLT, Hgb) were statistically treated in the following way:

The continuous variables which distributions did not have a deviation from the normal were presented as arithmetic average and standard deviation, while we used as average value and measure of dispersion for continuous variable which distribution significantly differed from normal the median and interquartal distribution. To confirm the statistical difference we used Student t-test for independent or dependent samples, as well as Mann-Whitney U test as non parametric alternative. The possibility of errors were accepted for $\alpha < 0.05$, and the difference between groups were accepted as statistical relevant for $p < 0.05$. P values that could not be showed with a 3 decimals digits, are shown as $p < 0.001$. The results obtained in this way are presented in two groups: A results before procedure (P1) and results after procedure (P2) and B are compared results between procedures (DN:SN). For the statistical analysis of obtained results the program SPSS for Windows was used (13.0, SPSS Inc, Chicago, Illinois, SAD) and Microsoft Excell (Office 2007, Microsoft Corporation, Redmond, WA, SAD).

RESULTS

In the following tables the values of hemocytes (HCT), leukocytes (WBC), platelets (Plt) and hemoglobin (Hgb) are given before (P1) and after separation (P2), the difference of these values and the comparison of the results between the procedures (DN:SN).

Table 2. Differences of observed values before (P1) and after (P2) separation

AparProc		\bar{X}	SD	t	p^{\ddagger}
AMDN	P1HCT	45.38	2.69	10.897	<0.001
	P2HCT	43.69	2.58		
	P1Hgb	15.44	1.00	13.225	<0.001
	P2Hgb	14.57	1.12		
	P1WBC	7.50	1.98	15.949	<0.001
	P2WBC	5.92	1.54		
	P1PLT	282.27	54.60	33.485	<0.001
	P2PLT	202.83	43.04		
AMSN	P1HCT	44.39	2.73	15.712	<0.001
	P2HCT	41.30	2.85		
	P1Hgb	15.45	0.87	16.651	<0.001
	P2Hgb	14.25	1.00		
	P1WBC	7.46	1.61	3.227	0.002
	P2WBC	7.07	1.88		
	P1PLT	281.83	51.32	27.776	<0.001
	P2PLT	208.37	36.93		

\ddagger t-test for dependent samples

Table 3. Differences for the observed parameters between the DN and SN groups

	AparProc		Test	p
	AMDN	AMSN		
P1HCT; $\bar{X}\pm SD$	45.38±2.69	44.39±2.73	t=3.010	0.003 [‡]
P1Hgb; $\bar{X}\pm SD$	15.44±1.00	15.45±0.87	t=0.053	0.958 [‡]
P1WBC; M (IR)	7.20 (2.57)	7.42(2.30)	Z=-0.438	0.662 [†]
P1PLT; $\bar{X}\pm SD$	282.27±54.60	281.83±51.32	t=0.069	0.945 [‡]
P2HCT; M (IR)	43.85 (3.70)	40.90 (3.10)	Z=-7.237	<0.001 [†]
P2Hgb; $\bar{X}\pm SD$	14.57±1.12	14.25±1.00	t=2.497	0.013 [‡]
P2WBC; M (IR)	5.61 (2.05)	6.80 (2.16)	Z=-5.462	<0.001 [†]
P2PLT; $\bar{X}\pm SD$	202.83±43.04	208.37±36.93	t=1.128	0.260 [‡]

[†]Mann-Whitney U test; [‡]Student t-test; $\bar{X}\pm SD$ = Arithmetic mean and standard deviation; M (IR) = Median and interquartile range

Results before procedure (P1) and results after procedure (P2).

Statistically significant lower values were observed of all researched parameters after separation for the donors on the equipment Amicus DN, and for donors on Amicus SN (Table 2).

B Comparison of results between the procedures (DN:SN).

The results of the statistic tests for the comparison of the variables before procedure (P1) for DN and SN procedure (Table 3):

A significant higher value of HCT before procedure was found in the AM DN group, in the researches of the other variables there were no significant differences. The results of statistical test for the comparison of variables after procedure (P2) procedure for DN and SN procedure (Table 3): A significant higher value of HCT and a significant higher level of Hgb, as well as a significant lower level of WBV after procedure in the AMDN group, while for the levels of PLT there were no significant differences.

DISCUSSION

In the group I, the value of hematocytes, the concentration of hemoglobines and the number of leukocytes and platelets on the Fenwal AMICUS equipment with the SN procedure before and after separation shows statistical significant differences. In all the observed parameters the values after separation, whether it is SN or DN, there is a statistical significant decrease of these values. The decrease of the value of these parameters after procedure is linked with the values the donor had before procedure. (Wollersheim et al. 2006, Several authors, as Patidar et al. 2013, Fontana et al. 2011, Altuntasa et al. 2008, Das et al. 2009, Bereta et al. 1998, Al-Raha et al. 2012, Chaundhary et al. 2009, Col et al. 2009, Vamvakas 2009, Fontana et al. 2006, Hans-Gert et al. 2013, Howard et al. 2005, also mention a decrease of the numbers of hematocytes, hemoglobine, leukocytes in the blood of the donor after the SN procedure on the Fenwal AMICUS equipment.

In Group II the values of hematocrites, the concentration of hemoglobine and number of leukocytes and platelets on Fenwal AMICUS equipment with the DN procedure, before and after separation shows statistical significant differences.

The conclusion that can be drawn from this statistical analysis is that within this group we have a statistical significant fall of the values of hematocrites, concentration hemoglobine, number of leukocytes and platelets after the procedure is completed. The decrease in number of platelets is expected since the aim of the procedure is to collect platelets. Other authors have come to results that confirms our results (Altuntasa et al. 2008, Das at al. 2009, BereTMA et al. 1998, Al-Raha et al. 2012, Chaundhary et al. 2009, Col et al. 2009, Vamvakas 2009, Hans-Gert et al. 2013).

After comparison of the Group I and II between 112 SN-IFCS separations and 180 DN-CFCS separations on Fenwal AMICUS equipment composed of 60 respondents on which were compared the values of hematocrites, the concentration of hemoglobine and number of leukocytes after plateletapheresis, the following results were obtained:

In the group of parameters (HCT, HgB, WBC, PLT) before separation (P1) we found that there is a statistical difference between the values of HCT (P1) between the DN and SN procedures. These difference even if small statistically exists. The rest of the parameters (Hgb, WBC, PLT) (P2) in SN and DN do not show an existing statistical significant difference. All of this shows that the group of donors that went through separation were well and in a unified way selected.

Comparing the parameters hematocrites and hemoglobine, we have a statistically significant difference in the decrease of these values after separation, (P2), that is there is a greater decrease of the values in the SN procedure than in the DN procedure. Because of the physiological link between these two values it is understandable that there is a decrease of the two values at the same time. The possible explanation of this decrease between DN and SN procedure is that in the SN procedure there is a greater hemodilution of the

blood of the donor. In difference to the SN procedure where there is a greater decrease in HCT and Hgb in the DN procedure we found a statistical greater decrease of WBC after procedure. The reason for this could be that in the SN procedure a bigger amount of WBC is returned to the donor together with the physiological solution and the erythrocytes.

The cause of this observation of decrease of the value of the observed parameters in specific procedure is not the subject of our research which is just to confirm if the decrease exists and if on the same equipment it differ according to the type of procedure used. Anyway the cause of these results should be researched specifically. Similar results in their work are mentioned by other authors Burgstaler et al. 1997, Das et al. 2009, Wagner 2012, Joseph Philip et al 2015.

Comparing the decrease in the values of platelet after procedure on Fenwal AMICUS equipment there is no statistical significant difference. The mentioned fact shows that on the same equipment, in this case Fenwal AMICUS, during plateletapheresis, the equipment equally efficiently from the blood of the donor collect platelets regardless on the procedure SN or DN, and the type of procedure statistically influences on the decrease of the values of hematocrits, the concentration of hemoglobine and number of leukocytes. To these same conclusions have also reached the following authors Patidar et al. 2013, Fontana et al. 2011, Altuntasa et al. 2008, Das et al. 2009, Bereta et al. 1998, Al-Raha et al. 2012, Chaundhary et al. 2009, Col et al. 2009, Vamvakas 2009, Fontana et al. 2006, Hans-Gert et al. 2013, Howard et al. 2005, Tendulkar & Rajadhyaksha 2009, Joseph Philip et al. 2015.

It is also important to remind that regardless of the confirmed decrease of the values of the observed parameters we did not have in a case that the observed parameters went below the physiological values after the procedure which tend to show the reliability and security for the donor when executing on of the procedures.

CONCLUSIONS

On the basis of the results we arrived to in this study we came to the following conclusions. A good selection of the donors is the main precondition and for the donor a sure platelet separation. In all observed separation of platelet, regardless of the separation there is a decrease of the value of hematocrits, the concentration of hemoglobins, number of leukocytes and platelets after a plateletapheresis. On the decrease of the value of the observed parameters the type of procedure has an influence that means DN-CFCS or SN-IFCS, continuous or discontinuous flow.

If the procedure is done according to the instructions and recommendations, and the maintenance is in accordance to the requirements of the manufacturer the potential health risks are reduced to their minimum and

the clinical consequences are irrelevant even in the case of several short platelet donations. Before choosing the procedure, DN-CFCS or SN-IFCS, all the parameters of the donor should be checked and it is essential to check once more all parameters of the donors before taking the decision on the type of procedure.

The concentration of platelets obtained after plateletapheresis will be in the future the main source of platelets for the treatment of the most difficult diseases.

The concentration of platelets obtained by apheresis is the best way to bridge the gap between the need of the patients with „rare“ blood groups and the possibilities of the transfusion centers. Regardless of the technological advances, before and after each separation control the hematocrits, hemoglobin, leukocytes and platelets in the donor's blood.

In practice, we often accept new technology and only observe the positive effects, ignoring the side effects, that actually are not dramatical, but can have a cumulative effect with time and that is why we should take them into consideration.

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Contribution of individual authors:

Elvedin Landžo, conception and design of the publication, literature searches and analyses, acquisition of data, interpretation of results, writing the first draft participate in drafting the article, execution of tables, approval of the final version

Josip Petrović, statistical analyses, participate in revising it critically for important intellectual content, approval of the final version.

Maja Karin, statistical analyses, participate in revising it critically for important intellectual content, approval of the final version.

Ivan Tomić, revising the manuscript, approval of the final version

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