Comparison Of Hemolysis Events And Hematocrit Quality In *Leucodepleted* (In Line) And *Leucodepleted* (Bedsite) Clinical Transfusions

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Abstract.
Blood transfusions no longer provide all the blood components but only provide the necessary blood components. We aim to determine whether there is a comparison of hemolysis events and hematocrit quality in leucodepleted (In Line) and leucodepleted (Bedsite) clinical transfusions. This study was an analytic study with a cross-sectional design to compare hemolysis events and hematocrit quality in leucodepleted (In Line) and leucodepleted (Bedsite) clinical transfusions. We obtained sample data in this study divided into 2 groups, namely the group with leucodepleted in line and the group with leucodepleted bedsite inclusion and exclusion criteria of 58 samples.We found that the incidence of hemolysis after leucodepleted (in line) was 0% and leucodepleted (bedsite) was 0.02%, where the standard percentage of blood hemolysis was <0.8%, the hemolysis incident after the production of the two products was very small. While the hematocrit before and after leucodepleted decreased by 35.91% in leucodepleted (in line) and 33.33% in leucodepleted (bedsite). There is a comparison of the incidence of leucodepleted (In Line) and leucodepleted (Bedsite) hemolysis. Where the incidence of leucodepleted (in line) hemolysis is not found, so the leucodepleted (in line) blood component has good quality. There is a comparison of the quality of leucodepleted (In Line) and leucodepleted (Bedsite) hematocrit.

Keywords: *Blood Transfusion, Blood Donors, Leukodepleted, Hemoglobin and Red Blood Cell.*

I. INTRODUCTION

Blood transfusion service is a health service effort in the form of giving blood/blood components to patients for the purpose of curing disease and/or choosing health. Blood transfusion services are carried out by the Blood Bank Unit at the Hospital [1]. Before blood is given to the patient, a pretransfusion examination is carried out. This pre-transfusion examination includes blood group examination and crossmatch. In addition, previously a serological examination was also carried out to test the eligibility of blood to be free from disease by the Indonesian Red Cross. [1]. There is an independent risk factor for adverse events to the patient when blood is transfused [2]. Increased mortality is always associated with blood transfusion [3], which can lead to infection and sepsis [4], when there is prolonged hospital stay and multi-organ system dysfunction is present [5].In today's developing era, blood transfusions no longer provide all the blood components but only provide the necessary blood components, for example, if there is a shortage of red blood cells, only red blood cells are given [6]. However, it should be noted that blood transfusions, on the other hand, can also cause transfusion reactions in the form of hemolytic reactions, febrile reactions, lung sensitivity reactions, anaphylactic allergic reactions, endotoxinemia, pulmonary edema, infection reactions and so on [7].Acute hemolytic reactions and delayed hemolytic reactions occur due to increased destruction of red blood cells by blood transfusions [6][7]. Within 24 hours after blood transfusion an acute type reaction will occur due to erythrocyte ABO incompatibility which triggers IgG/IgM antibodies with complement activation causing intravascular hemolysis and rhesus incompatibility which triggers IgG antibodies against rhesus factor causing extravascular hemolysis [7].

While the delayed type of transfusion reaction occurs 3-10 days after the transfusion, it is usually caused by the presence of low levels of antibodies against minor antigens of erythrocytes. Upon exposure to antigenic cells, these antibodies rapidly increase in level and cause extravascular hemolysis [8].Acute non-hemolytic transfusion reactions vary up to 38% of all platelet and red blood cell transfusions. Non-hemolytic fever reactions often occur 1.7% -30% and allergic reactions 1% -3% [9]. The incompatibility of 1:76,000 for hemolytic transfusion reactions and the 1:40,000 for ABO incompatibility. Slow-type hemolytic transfusion reaction 1:18,000. Non-hemolytic fever reactions 0.1-1% and allergic reactions (urtica) 1-3% [10].The use of washed erythrocyte (WE) and leucodepleted-PRC (LD-PRC) as an effort to minimize

transfusion reactions by modifying the manufacture of blood components and blood products because blood transfusions only contain a small number of leukocytes and plasma. [11]. Use of apheretic blood products from a single donor and leucodepleted blood products will result in insufficient transfusion reactions. [12].

To make the life span of blood components longer, *Leucodepleted* is a filtration method that is 99.99% efficient and practically removes leukocytes. [11]. The European standard of *leucodepleted* must be the number of leukocytes in the blood or blood components to be transfused up to a minimum of < 1x106 leukocytes/unit [12].There are two methods of reducing the number of leukocytes, namely reducing the number of leukocytes before storage (pre-storage leucodepletion) and reducing the number of leukocytes after storage (post storage leucodepletion). To increase the effectiveness of leukocyte filtration, currently the bedsite leucocyte filter can be used closed on blood components prior to storage or immediately after storage by connecting the bedsite leucocyte filter to the satellite bag using a sterile connecting device (SCD). While leucodepleted in-line is a method in which blood products are directly filtered in-line, undergo pre-storage and are centrifuged to obtain leucodepleted (in-line) [11].The plasma protein component in the leucodepleted product (in-line) was 99.97% and the hemolysis after the product was manufactured was very small (<0.8%) [11]. Leucodepleted (bedsite) there was a significant decrease in the number of leukocytes with an average of 2.26 [11]. The quality of the hematocrit plays an important role in blood transfusion. The hematocrit level in the leucodepleted (In Line) was 76.9% while the leucodepleted (bedsite) was 71.02% [11][13].

II. METHODS

The study variables consisted of the dependent variables, namely leucodepleted (in line) and leucodepleted (bedsite) and the independent variables were hemolysis events and hematocrit quality. Design The study was an analytic study with a cross-sectional design to compare hemolysis events and hematocrit quality in leucodepleted (in line) and leucodepleted (bedsite) clinical transfusions.Research Place at the Indonesian Red Cross Blood Transfusion Unit in Medan City. The time of the study was conducted from August 2019 to September 2019 for sampling and examination of hemolysis events and hematocrit quality at *Leucodepleted* (in line) and *Leucodepleted* (bedsite). Data analysis was carried out in September 2019. The population of this study were all donors who came to UTD PMI Medan City.The sample in this study was divided into 2 groups, namely the group with leucodepleted in line and the group with leucodepleted bedsite inclusion and exclusion criteria. Inclusion criteria (Willing to be a respondent by signing an informed consent, Patients who come to UTD PMI Medan city, Patients who meet the criteria for blood donors and whole blood stocks that are < 24 hours) then Exclusion criteria (Suffering from infectious diseases such as hepatitis A and B, HIV, syphilis, CMV and not willing to participate in the study).

This research is included in the categorical-numerical analytic research thus, the sample size formula is as follows:

$$n = \left[\frac{Z\alpha\sqrt{2PQ} + Z\beta\sqrt{P_1Q_1 + P_2Q_2}}{P_1 - P_2}\right]^2$$

n = number of samples
Alpha (α) = Type one error is set at 5%, one-way hypothesis so Zα = 1.96
Beta (β) = Type two error, set at 20%, then Zβ = 0.84
P2 = proportion in the standard group of 0.5 (reference)
Q2 = 1 – 0,5 = 0,5
RR = 1,5
P1 = RR x P2
P1= 1,5 x 0,5 = 0,75
Q1 = 1 – P1 = 1 – 0,75 = 0,25
P1 – P2 = 0,75 – 0,50 = 0,25
P = (P1 + P2)/2 = (0,75 + 0,50)/2 = 0,63
Q = 1 – P = 1 – 0,63 = 0,37
Based on the calculation results above, it was found that the number of samples was 58.

Sampling used a consecutive sampling technique from a random population as long as it met the inclusion and exclusion criteria. The study used primary data including collecting respondent data, hemolysis events and hematocrit quality at *Leucodepleted* (in line) and *Leucodepleted* (bedsite).

How Research Works

As for the workings of this research, the researcher first obtained approval from the ethical committee. then as an initial screening, the subject must meet the requirements as a donor, namely as follows: Minimum age 17 years. First-time donors > 60 years of age and repeat donors > 65 years of age can become donors with special attention based on medical considerations of health conditions. Have a minimum body weight of 45 kg with a systolic blood pressure of 90-160 mmHg and a diastolic of 60-100 mmHg. The difference between systolic and diastolic is more than 20 mmHg. Have a pulse rate of 50-100 times per minute and regular and body temperature of 36,5 – 37,5^{0}C and hemoglobin level of 12,5 – 17 g/dl and the interval since the last blood donation is 2 months.

The next stage is to take 450 ml of donor blood (whole blood) and immediately perform leucodepleted in line with a sterile connector. The next step is to check the hematological parameters (routine blood) with the Sysmex® XP 300, where approximately 4 ml of blood will be taken for each sample for haematological examination using the Radio Frequency (RF) / Direct Count (DC) detection method to count erythrocytes and platelets. flow cytometer method with a semiconductor laser to count the number of *leukocytes, neutrophils, reticulocytes* and *platelets*. Then the next step is to *Leucodepleted* bedsite on whole blood blood stock with storage time < 24 hours with a sterile connector, and Imugard III RC filter made of 1-2µ microporus polyurethane which works mechanically. The blood bag is connected to the filter and the satellite bag using a sterile connector. Then the next step is to examine the incidence of *Leucodepleted* in-line and *Leucodepleted* bedsite hemolysis using the HemoCue® tool.

Data Analysis Method

Data processing is carried out in the following stages: edit data to avoid errors or the possibility of an unfilled questionnaire. Coding the data to facilitate the data entry process, each answer is given a code and a score. Entering data after being coded and checking and repairing before data analysis.Data analysis was carried out with homogeneity and normality tests to determine the difference test to be used. If the data is normally distributed, a parametric sign of difference test is performed, whereas if the data is not normally distributed, a non-parametric sign of difference test is performed.

III. RESULT AND DISCUSSION

The results of the study showing a comparison of hemolysis events and hematocrit quality in *Leucodepleted* (in line) and *Leucodepleted* (bedsite) obtained haematological parameters (routine blood) before and after *Leucodepleted*.

Table 1. Hematological Results (Routine Blood)

No	In Line (%)				Bed Side (%)			
	Blood ≤ 24 hours Before *Leucodepleted*		Leukodeplted		Blood Store ≤ 24H Before *Leucodepleted*		Leukodepleted	
	Hemolisis	Hematokrit	Hemolisis	Hematokrit	Hemolisis	Hematokrit	Hemolisis	Hematokrit
1	0	40.2	0	39.6	0.253	52.7	0.023	35.9
2	0	41.1	0	39.2	0.046	40.1	0.003	28.3
3	0	39.3	0	38.8	0.161	36.4	0.128	32.7
4	0	34.2	0	32.5	0.303	36.7	0.229	44.1
5	0	34.1	0	32.9	0.058	33.6	0	25
6	0	38.6	0	37.1	0.126	32.7	0	30.8
7	0	35.8	0	34.9	0	34.4	0	28.6
8	0	33.9	0	33.2	0.109	36.6	0	28.8
9	0	34.9	0	33.3	0	34.4	0	28
10	0	39.1	0	37.4	0.045	39.2	0.042	37.2
11	0	36.6	0	35.6	0.09	40.4	0.021	30.1
12	0	32.8	0	32.5	0.118	34.5	0.002	25.7
13	0	35.7	0	35.7	0.191	39.2	0	36.2

14	0	40.2	0	38	0.180	40.6	0.032	30.5
15	0	38.9	0	37.2	0.191	46.1	0.096	53.9
16	0	34.7	0	34.3	0.066	48.5	0.052	29
17	0	37.2	0	35.1	0.170	50.1	0	40.5
18	0	33.8	0	33.2	0.052	36.5	0.052	26.8
19	0	42.2	0	41.0	0.167	48.1	0.023	28.8
20	0	38.0	0	37.5	0.100	37.9	0.092	30.1
21	0	33.3	0	32.7	0.125	63.0	0	45.4
22	0	34.2	0	33.8	0.250	52.2	0	31.7
23	0	36.7	0	34.6	0.043	40.4	0.023	35.4
24	0	40.2	0	38.7	0.159	36.6	0.032	30.0
25	0	34.9	0	34.6	0.300	36.1	0	38.2
26	0	33.8	0	32.4	0.056	33.3	0	29.3
27	0	37.1	0	35.1	0.123	32.5	0	30.7
28	0	39.8	0	34.7	0.123	34.1	0	35.8
29	0	36.9	0	34.2	0.106	36.2	0.012	31.6
30	0	34.1	0	32.1	0.118	34.4	0.123	29.0
31	0	34.5	0	33.9	0.042	39.2	0.054	30.8
32	0	41.8	0	40.0	0.124	38.4	0.021	33.5
33	0	33.7	0	32.6	0.119	34.6	0	30.9
34	0	37.3	0	35.7	0.190	39.0	0	30.2
35	0	36.2	0	35.2	0.178	40.4	0	32.2
36	0	34.8	0	33.8	0.188	46.0	0	37.1
37	0	35.2	0	34.8	0.064	48.3	0	31.7
38	0	35.1	0	33.3	0.165	50.0	0	42.1
39	0	41.2	0	39.8	0.057	36.3	0	31.2
40	0	37.9	0	37.5	0.165	46.1	0.042	32.2
41	0	37.3	0	36.9	0.108	31.7	0.032	28.5
42	0	39.3	0	38.4	0.123	60.2	0	44.1
43	0	37.9	0	37.6	0.055	32.5	0	35.6
44	0	32.2	0	33.6	0.123	34.2	0.021	32.3
45	0	37.4	0	36.2	0.133	36.4	0.036	33.9
46	0	36.7	0	35.5	0.105	34.2	0	30.1
47	0	33.3	0	32.6	0.132	39.0	0	33.1
48	0	35.2	0	34.8	0	41.2	0	32.8
49	0	34.4	0	33.9	0.110	33.1	0.066	29.5
50	0	39.1	0	37.7	0.115	37.1	0.023	34.3
51	0	33.2	0	32.1	0.198	41.3	0.024	30.6
52	0	41.4	0	40.8	0.185	46.0	0	34.5
53	0	33.6	0	32.5	0.188	47.3	0	40.0
54	0	38.0	0	37.1	0.068	49.3	0.012	39.5
55	0	35.9	0	35.6	0.166	35.6	0	33.1
56	0	40.9	0	40.7	0.050	47.6	0.034	37.3
57	0	44.2	0	43.3	0.165	37.5	0	32.6
58	0	45.8	0	45.2	0.102	62.1	0	41.1

Table 2. Hematological Parameters (Routine Blood)

Parameters	**Leukodepleted (*In Line*) (n=58)**		**Leukodepleted (*Bedsite*) (n=58)**	
	Before	**After**	**Before**	**After**
Hemoglobin (g/dl) (Avg ±SD)	13,08 ± 1,22	12,24 ± 1,51	13,92 ± 1,71	11,54 ±1 ,71
Hematokrit (%) (Avg±SD)	36,99 ± 3,05	35,91 ± 2,96	40,76 ± 7,55	33,33 ± 5,41
Eritrosit (x10/µL) (Avg±SD)	4,57 ± 0,53	4,12±0,54	4,75 ± 0,81	4,14 ± 0,88
Leukosit (x10/µL) (Avg±SD)	0 ± 0	0 ± 0	6,53 ± 1,44	0 ± 0
Hemolisis (Avg±SD)	0 ± 0	0 ± 0	0,12 ± 0,06	0,02 ± 0,04

The results of routine blood tests before leucodepleted found that there was no hemolysis in leucodepleted (in line) and the average hematocrit level in leucodepleted (in line) was 36.99% while in leucodepleted (bedsite) the average hemolysis was 0.12 and the average hematocrit level on leucodepleted

(bedsite) of 40.76%. Routine blood examination after leucodepleted found that there was no hemolysis in leucodepleted (in line) and the average hematocrit level in leucodepleted (in line) was 35.91% while in leucodepleted (bedsite) the average hemolysis was 0.02 and the average hematocrit level in leucodepleted (bedsite) of 33.33%.

Table 3. Normality and Homogeneity Test Results

Variable	Normality test	Information
Hemolysis Events	0,000	Not normally distributed
Hematocrit Quality	0,000	Not normally distributed

The results of the non-parametric test, namely the Kruskall Wallis Test provided that the above results show a normality test p = 0.000 (p <0.05) so that the data is not normally distributed and when the homogeneity test is carried out it is obtained p = 0.000 (p <0.05), i.e. the data is not homogeneous. Results of data analysis regarding the comparison of hemolysis and hematocrit quality to *Leucodepleted* (in line) and *Leucodepleted* (bedsite).

Table 4. Comparison of hemolysis and hematocrit events in *Leucodepleted* (In Line) and *Leucodepleted* (Bedsite) with the Kruskall Wallis Test

Variabel	N	Avg ± S.D	P
Hemolysis Events	58	0,01 ± 0,03	0,000
Hematocrit Quality	58	34,62 ± 4,53	0,000

The results obtained p = 0.000 (p <0.05) then H0 was rejected so it can be concluded that there is a comparison of hemolysis and hematocrit events in leucodepleted (in line) and leucodepleted (bedsite).

DISCUSSION

The results of the study found that the incidence of hemolysis after leucodepleted (in line) was 0% while leucodepleted (bedsite) was 0.02%. This shows that the incidence of hemolysis is small. There is hemolysis in blood transfusions describing a damage or disruption of the integrity of the blood cell membrane which causes the release of hemoglobin [14][11].The results of the analysis showed that the value of p = 0.000 (p <0.05) showed a difference between the incidence of leucodepleted (in line) and leucodepleted (bedsite) hemolysis. Leucodepleted (in line) hemolysis results were not found. So that leucodepleted (in line) blood components have good quality against hemolysis events. The leucodepleted component (in line) has good quality and is safely given to clinical transfusion patients and also to multiple transfusion patients with different indications [11]. Leucodepleted (bedsite) shows the incidence of hemolysis is 0.02%.

Hemolysis can be caused by several factors, namely blood processing, improper storage conditions, bacterial hemolysin, antibodies that cause complement lysis, damage to cell membranes, or abnormalities in donor blood [12]. Based on these factors, leukocyte removal is preferable if the time between blood collection and leucodepleted is shortened. This occurs because, during storage, leukocytes degranulate, fragment or die releasing their contents which can cause transfusion reactions such as hay fever and allergies. Cytokines in particular such as IL-8 which accumulate during storage have been implicated in several bedsite filtration failures thereby triggering blood hemolysis [13][15].The results showed that the hematocrit value decreased before and after leucodepleted either in line or bedsite. Data analysis showed a value of p = 0.000 (p <0.05) so that there was a difference in hematocrit for *Leucodepleted* (in line) and *Leucodepleted* (bedsite). The *Leucodepleted* component has a decreased hematocrit. This happens because in the process of making *Leucodepleted* there are anticoagulants so that the blood will become dilute and the hematocrit level will decrease [11][16].

IV. CONCLUSION

Based on the results of this study, the hematological parameters after leucodepleted (in line) were 0% and leucodepleted (bedsite) was 0.02%, where the standard percentage of blood hemolysis was <0.8%, the occurrence of hemolysis after the production process of the two products was very small. While the hematocrit before and after leucodepleted decreased by 35.91% in leucodepleted (in line) and 33.33% in leucodepleted (bedsite).Then also obtained from the results of this study There is a comparison of the incidence of leucodepleted (In Line) and leucodepleted (Bedsite) hemolysis. Where the incidence of

leucodepleted (in line) hemolysis is not found, so the leucodepleted (in line) blood component has good quality. Then also obtained from the results of this study There is a comparison of the quality of leucodepleted (In Line) and leucodepleted (Bedsite) hematocrit. It is recommended to continue to carry out hemolysis examinations in patients who will do *Leucodepleted* (in line) or *Leucodepleted* (bedsite). To reduce the incidence of hemolysis and transfusion reactions, leucodepleted blood components can be used in clinical transfusions. It is better to do further research regarding the use of *Leucodepleted* (in line) and *Leucodepleted* (bedsite) in patients who have repeated blood transfusions.

V. ACKNOWLEDGMENTS

The author thanks the Blood Transfusion Unit at the Indonesian Red Cross in Medan City for supporting this research, to Prima Indonesia University and to the Head of the biomedical science master's program, Faculty of Medicine, UNPRI. We also thank the supervising lecturers for their invaluable advice.

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