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Differences in Hemoglobin Levels 6 Hours and 24 Hours After Packed Red Cells Transfusion

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ABSTRACT

The hemoglobin examination instructed by Dr. Sardjito General Hospital attending doctors was 6 hours after the PRC transfusion. It is recommended that a hemoglobin examination be carried out 24 hours after transfusion to determine the patient's hemoglobin after complete equilibrium occurs. This study aimed to determine the mean increase in Hb levels 6 hours and 24 hours after PRC transfusion and to examine the difference in Hb levels 6 hours and 24 hours after PRC transfusion, respectively, compared with baseline Hb levels in non-hematological malignancy patients. This prospective analytic observational study examined hemoglobin at 6 hours and 24 hours after PRC transfusion. The differential test between delta Hb levels 6 hours after PRC transfusion compared to baseline Hb levels and delta Hb levels 24 hours after PRC transfusion compared to baseline Hb levels were analyzed using paired T-test. There was a statistically significant difference between baseline Hb levels and Hb levels 6 hours after PRC transfusion ($p<0.0001$) and a statistically significant difference between baseline Hb levels and Hb levels 24 hours after PRC transfusion ($p<0.0001$). The differential test between delta Hb levels 6 hours after PRC transfusion compared to baseline Hb levels and delta Hb levels 24 hours after PRC transfusion compared to baseline Hb levels obtained a statistically significant difference ($p=0.0024$). The mean increase in hemoglobin was 1.76+0.78 g/dL 6 hours after PRC transfusion and 1.9+0.78 g/dL 24 hours after PRC transfusion. There were differences in delta Hb levels 6 hours and 24 hours after PRC transfusion, respectively, compared with baseline Hb levels in non-hematological malignancy patients, which were statistically significant but not clinically significant.

Keywords: Non-hematological malignancy, anemia, hemoglobin, transfusion, PRC

INTRODUCTION

Cancer or malignancy is the second leading cause of death in the world. According to data from GLOBOCAN, in 2020, there were 19.3 million new cases, with a total death of 9.9 million people in the world. Asia is the most common place for malignancy (49.3%) and deaths from malignancy (58.3%). In Indonesia, there were 396,914 new cases, with number of deaths of as many as 234,511 people, with the most cases being breast, cervical, lung, colorectal, and liver malignancies.[1]

Anemia is a complication that often occurs in patients with malignancy. According to the European Cancer Anemia Survey (ECAS), anemia occurs in 39% of malignancy cases, and 67% of patients have chemotherapy-induced cytopenia.[2] Anemia caused by malignancy may arise as a direct effect of malignancy (due to tumor infiltration into the bone marrow and/or proinflammatory cytokines that affect the process of erythropoiesis) and/or due to treatment of the malignancy itself.[3]

Packed Red Cells (PRC) transfusion can increase hemoglobin (Hb) and hematocrit (Hct) rapidly, making it an ideal choice to correct anemia immediately. Transfusion of 1 unit of PRC (300 mL) is estimated to increase the Hb level by 1 g/dL in normal adult patients.[4] Anemia in malignancy patients can be treated by transfusion, and 15% of non-hematological malignancy patients are treated with transfusion.[5]

It is implied that the blood volume increases immediately after the transfusion and does not return to normal for 24 hours. Equilibrium of hemoglobin levels after transfusion is estimated to take about 24 hours, but some studies have shown that earlier measurements reflect steady-state values in patients without bleeding.[6] After PRC transfusion in stable patients, equilibrium of hemoglobin occurs over time before hemoglobin stability is achieved. Although there is no consensus on the appropriate time for post-transfusion hemoglobin examination, several studies suggest early post-transfusion hemoglobin examination.[7-9]

Hemoglobin monitoring after PRC transfusion is essential to be done in assessing the success of a transfusion. The time factor of Hb examination after transfusion must be established to judge the success

of a blood transfusion.[10] Based on observation in the inpatient ward of Dr. Sardjito General Hospital, the time for checking Hb after PRC transfusion as instructed by attending doctors was 6 hours after PRC transfusion, but this time duration has not been included in the standard operating procedure of the service. Early studies of changes in hemoglobin after blood transfusion recommended that post-transfusion hemoglobin tests be performed 24 hours after transfusion to determine the patient's hemoglobin after complete equilibrium occurred.[7] This study aimed to determine the mean increase in Hb 6 hours and 24 hours after PRC transfusion and to examine the difference in Hb 6 hours and 24 hours after PRC transfusion, respectively, compared with baseline Hb levels in non-hematological malignancy patients.

METHODS

This was a prospective analytic observational study with hemoglobin examination 6 hours and 24 hours after PRC transfusion. This study was conducted at the Integrated Laboratory Installation, Inpatient Installation I, and the Medical Records Installation of Dr. Sardjito General Hospital, Yogyakarta. The study samples were collected from February to June 2021.

The study subjects were adult non-hematological malignancy patients who received PRC transfusions at the Inpatient Installation I Dr. Sardjito General Hospital, Yogyakarta, who met the inclusion and exclusion criteria. Inclusion criteria included non-hematological malignancy patients who were hospitalized, age 18 years, Hb levels <7 g/dL, Hb levels 7-10 g/dL with comorbidities, and patients with transfusion plans to undergo chemotherapy procedures. Exclusion criteria included evidence of active bleeding and proof of a hemolytic process from any cause before PRC transfusion. Study subjects were required to sign an informed consent. Patients were considered to drop out of the study if they died before the hemoglobin test series after the PRC transfusion was completed if the hemoglobin test series after the PRC transfusion was not performed for any reason, and if evidence of a hemolytic process from the peripheral blood smear examination after the PRC transfusion was performed. In this study, the security of the analytical method was carried out by conducting calibration tests, accuracy tests, and precision tests before carrying out laboratory examinations.

This study used ethical clearance issued by the Medical and Health Research Ethics Committee (MHREC), Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada/Dr. Sardjito General Hospital (Ref. No. KE/FK/0033/EC/2021).

Characteristic data of study subjects are displayed descriptively in the mean and standard deviation if the data distribution was normal or median and the minimum-maximum value if the data distribution was not normally distributed. The normality of the study subject data was carried out using the Kolmogorov-Smirnov test. The differential test between the delta Hb levels 6 hours after the PRC transfusion compared to the baseline Hb levels and the delta Hb levels 24 hours after the PRC transfusion compared to the baseline Hb levels were analyzed using a paired T-test. Analysis of study data using Medcalc version 13. Study data was presented in the form of tables and graphs. The level of statistical significance was determined namely $p < 0.05$.

RESULTS AND DISCUSSIONS

One hundred seventy-two non-hematological malignancy patients met the inclusion criteria, while 61 patients with active bleeding conditions were excluded. One hundred eleven non-hematological malignancy patients met the inclusion and exclusion criteria. A total of 59 patients dropped out of this study. Twenty-four patients were discharged before the 24-hour post-PRC transfusion Hb examination was performed, two patients died before the 24-hour post-PRC transfusion Hb examination was performed, 21 patients received an additional PRC transfusion before the 24 hours post-PRC transfusion Hb examination was performed, and evidence of hemolytic process was found in 12 patients from peripheral blood smear examination after PRC transfusion. The remaining 52 patients were study subjects for Hb examination 6 and 24 hours post-PRC transfusion.

In this study, 12 patients dropped out because there was evidence of a hemolytic process with fragmentocyte and spherocyte cell images found on peripheral blood smear examination 6 hours after PRC transfusion. The clinical symptom of hemolytic reaction that appears in the patient is fever. When fever occurs as a result of a hemolytic transfusion reaction, it is most often associated with a transfusion of ABO-incompatible blood.[11]

The data on the basic characteristics of the study subjects are displayed descriptively in the mean and standard deviation if the data distribution is normal or median and the min-max value if the data distribution is not normal. The normality of the data was carried out using the Kolmogorov-Smirnov test. The basic characteristics of the subjects of this study are presented in Table 1.

Table 1. Basic characteristics of study subjects (n=52)

Variable	n (%)	Mean±SD	Median (Min-Max)
Age (years)		50.98±12.63	
Gender			
Male	25 (48.1)		
Female	27 (51.9)		
Body weight (kg)			50 (27-94)
Height (cm)		159.39 ± 8.82	
Body surface area (m^2)			1.5 (1.08-2.05)
Estimated blood volume (L)			3.51 (1.76-7.05)
History of heart failure			
Yes	3 (5.8)		
No	49 (94.2)		
Use of diuretics			
Yes	4 (7.7)		
No	48 (92.3)		
Types of malignancy			
Rectum	11 (21.2)		
Lungs	6 (11.5)		
Ovaries	5 (9.6)		
Urinary bladder	4 (7.6)		
Cervix	4 (7.6)		
Liver	3 (5.8)		
Skin	3 (5.8)		
Colon	2 (3.9)		
Breast	2 (3.9)		
Nasopharynx	2 (3.9)		
Endometrium	2 (3.9)		
Pancreas	2 (3.9)		
Colorectal	1 (1.9)		
Muscle	1 (1.9)		
Bone	1 (1.9)		
Thymus	1 (1.9)		
Testicles	1 (1.9)		
Uterus	1 (1.9)		
Malignancy stadium			
I	2 (3.8)		
II	20 (38.5)		
III	5 (9.6)		
IV	25 (48.1)		
Chemotherapy cycle			
1	15 (28.8)		
2	16 (30.8)		
3	10 (19.2)		
4	7 (13.5)		
≥ 5	4 (7.7)		
Splenomegaly	0 (0)		
Number of PRC (units)			2 (1-3)
Transfusion duration per PRC unit (minutes)			180 (90-225)

Body surface area is a better indicator of metabolic mass than body weight because it is less affected by abnormal adipose mass. The most widely used body surface area calculation is the Du Bois formula (BSA = 0.007184 x height (cm)0.725 x weight (kg)0.425), which is equally effective in estimating body fat in obese and non-obese patients, which body mass index cannot.[12]

Estimation of blood volume estimates intravascular blood by considering the patient's weight, height, and gender. Blood volume per kilogram varies by gender and age, with the mean blood volume per kilogram being higher in newborns than adults. Estimated Blood Volume (EBV) = 65 mL x weight (kg) for adult females or 75 mL x weight (kg) for adult males.[13]

History of heart failure and use of diuretics were found in 5.8% and 7.7% of subjects before the transfusion, respectively. Transfusion should be done carefully to avoid excess fluid volume, which

Table 2. Characteristics of the baseline laboratory parameters of study subjects (n=52)

Parameter	Mean±SD	Median (Min-Max)
Hemoglobin (g/dL)	8.34±1.13	
Hematocrit (%)	26.01±3.39	
Erythrocytes ($x10^6$/µL)	3.16±0.47	
Leukocytes ($x10^3$/µL)		8.12 (0.76-79.46)
Platelets ($x10^3$/µL)	298,83±168,27	
MCV (fL)	83±8.16	
MCH (pg)		26.95 (16.9-36.3)
MCHC (g/dL)		32.25 (27-36.8)
RDW-CV (%)	16.67±2.28	

can lead to increased mortality in CHF patients with anemia. Hypervolemia is associated with an increased risk of death because B-type natriuretic peptide, a hormone produced by the heart that is closely correlated with left ventricular end-diastolic pressure, is an independent predictor of survival in CHF patients.[14] Premedication of diuretics for patients receiving blood transfusions is common, especially in patients at high risk of fluid overload or pulmonary edema. Complications of Transfusion-Associated Circulatory Overload (TACO) are thought to be preventable with pre-transfusion diuretic therapy.[15]

The most common types of non-hematological malignancies in this study were rectum (21.2%), lung (11.5%), and ovaries (9.6%). The incidence of anemia in malignancy varies depending on the type, stage, and duration of illness, treatment regimen used, and presence or absence of infection. Patients with tumor types who reported a high ratio of anemia included lung cancer (52%) or ovarian cancer (51%).[16]

Anemia can occur in 90% of patients during chemotherapy, and cancer therapy often results in loss, destruction, and decreased production of erythrocytes, all of which lead to anemia.[3] The ECAS showed that anemia increased from 19.5% in the first cycle of chemotherapy to 46.7% after the fifth cycle. Other risk factors for developing chemotherapy-associated anemia include low hemoglobin levels, transfusion within the previous six months, radiotherapy >20% of the skeleton, previous myelosuppressive chemotherapy, and comorbidities such as chronic inflammatory disease.[4]

There were no study subjects with splenomegaly. The prevalence of splenic metastases in a large population with cancer ranges from 2.3-7.1%. Several theories have demonstrated the resistance of the splenic parenchyma to metastases, including the ability of the splenic capsule to form a physical barrier, the angular and undulating appearance of the splenic artery, and the immunological defense of the spleen against neoplastic cells.[17]

In this study, there were seven patients with Hb levels < 7 g/dL, 28 patients with Hb levels 7-10 g/dL with comorbidities, and 17 patients with transfusion plans to undergo chemotherapy procedures. The patients' leukocyte counts varied; two had leukocytes >50,000/L. The leukemoid reaction was defined as a leukocyte count of more than 50,000/L with a predominance of neutrophil precursors (Table 2). Although it can occur in severe infections, the leukemoid reaction can also present as a paraneoplastic syndrome in patients with various types of cancer.[18]

In Figure 1, a comparison between the baseline, 6-hour, and 24-hour Hb levels after PRC transfusion was carried out in 52 study subjects. In the Hb 6 hours after the PRC transfusion group, the lowest value was 6.7 g/dL, and the highest value was 12.2 g/dL, while in the Hb 24 hours after the PRC transfusion group, the lowest value was 6.9 g/dL and the highest value was 12.8 g/dL. The similarity of the results between Hb 6 hours and Hb 24 hours after PRC transfusion was indicated by a dot that coincided between Hb 6 hours and Hb 24 hours after PRC transfusion; it was concluded that there was no clinically significant difference between Hb levels 6 hours and Hb 24 hours after PRC transfusion in non-hematological malignancy patients.

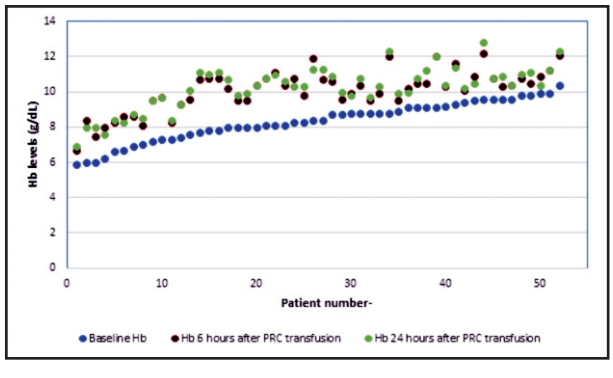

Figure 1. Comparison of Hb levels at baseline, 6 hours, and 24 hours after PRC transfusion

Table 3. The difference in Hb levels 6 hours after the PRC transfusion compared to baseline Hb levels, the difference in Hb levels 24 hours after the PRC transfusion compared to baseline Hb levels, and the difference of delta Hb levels 6 hours and 24 hours after PRC transfusion, respectively, compared with baseline Hb levels

Parameter	(Mean±SD)	p
Baseline Hb levels (g/dL)	8.34±1.13	
Hb levels 6 hours after PRC transfusion (g/dL)	10.09±1.2	< 0.0001*
Hb levels 24 hours after PRC transfusion (g/dL)	10.23±1.23	< 0.0001**
Δ Hb levels 6 hours after PRC transfusion (g/dL)	1.76±0.78	0.0024***
Δ Hb levels 24 hours after PRC transfusion (g/dL)	1.9±0.78	

* Differential test of Hb levels 6 hours after PRC transfusion compared to baseline Hb levels
** Differential test of Hb levels 24 hours after PRC transfusion compared to baseline Hb levels
*** Differential test of delta Hb levels 6 hours and 24 hours after PRC transfusion, respectively, compared with baseline Hb levels

In this study, the mean+SD baseline Hb levels, Hb 6 hours after PRC transfusion, and Hb 24 hours after PRC transfusion, respectively, were 8.34+1.13 g/dL; 10.09+1.2 g/dL; 10.23+1.23 g/dL. There was a statistically significant difference between baseline Hb levels and Hb levels 6 hours after PRC transfusion ($p < 0.0001$) and a statistically significant difference between baseline Hb levels and Hb levels 24 hours after PRC transfusion ($p < 0.0001$). Hb levels 24 hours after PRC transfusion were reported to be higher than Hb levels 6 hours after PRC transfusion (10.23+1.23 g/dL vs. 10.09+1.2 g/dL) (Table 3).

From the results of baseline Hb levels, Hb 6 hours after PRC transfusion, and Hb levels 24 hours after PRC transfusion, the delta results of Hb levels 6 hours after PRC transfusion compared to baseline Hb levels were 1.76+0.78 g/dL while the delta Hb levels 24 hours after PRC transfusion compared to baseline Hb was 1.9+0.78 g/dL. In Table 3, a differential test was carried out between the delta Hb levels 6 hours and 24 hours after PRC transfusion, respectively, compared to the baseline Hb levels; there was a statistically significant difference ($p=0.0024$).

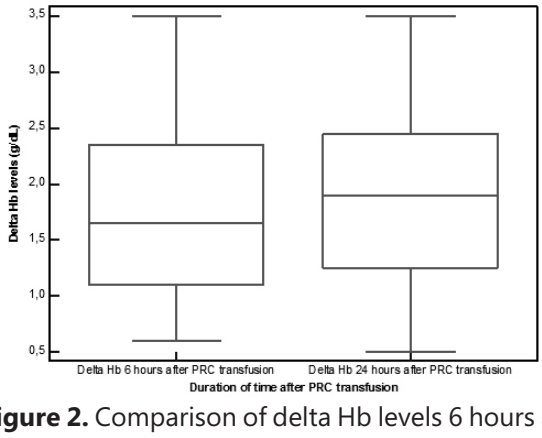

Figure 2. Comparison of delta Hb levels 6 hours and 24 hours after PRC transfusion, compared with baseline Hb levels, respectively

Figure 2 compares the delta Hb levels 6 and 24 hours after PRC transfusion with the baseline Hb levels in 52 study subjects using a box plot diagram. In the delta Hb levels 6 hours after PRC transfusion compared to the baseline Hb levels, the lowest value was 0.6 g/dL. The highest value was 3.5 g/dL, while in the delta Hb levels 24 hours after PRC transfusion compared to the baseline Hb levels, the lowest value was 0.5 g/dL, and the highest value was 3.5 g/dL. There were similar results between the delta Hb levels 6 hours and 24 hours after PRC transfusion, respectively, compared with the baseline Hb levels, it was concluded that there was no clinically significant difference between the delta Hb levels 6 hours and 24 hours after PRC transfusion, respectively compared with baseline Hb levels, in non-hematological malignancy patients.

Different literatures state that a 1 g/dL increase in hemoglobin is expected per unit of blood transfused. After the transfusion, hemoglobin equilibrium levels take about 24 hours, but little supporting evidence is found. The ability to rapidly determine elevated hemoglobin levels after transfusion is essential in managing outpatients and acutely ill patients.[9]

A patient is clinically said to be anemic if the hemoglobin or hematocrit is below the lower limit of 2 Standard Deviations (-2SD) or the 95% confidence interval for the normal population. This definition of anemia has an impact that 2.5% of normal individuals are classified as anemic.[19] In this study, the delta Hb levels 6 hours after PRC transfusion compared to the baseline Hb levels were 1.76+0.78 g/dL, while the delta Hb levels 24 hours after PRC transfusion compared to baseline Hb levels were 1.9+0.78 g/dL. The difference between the delta Hb levels 6 hours after PRC transfusion compared to the delta Hb levels 24 hours after PRC transfusion was 0.14 g/dL, indicating that it was not clinically significant, for example, between Hb 10.0 g/dL compared to 10.14

g/dL. The differential test between delta Hb levels 6 hours and 24 hours after PRC transfusion, respectively compared with the baseline Hb levels, found a statistically significant difference (p=0.0024) but not clinically important. In the study of Rajkumar *et al.*, hemoglobin was re-examined in patients who were given a transfusion of at least 1 unit of blood. The mean change in hemoglobin from first to second was 0.05 g/dL (95% CI 0.03-0.08), from 10.2±2.1 g/dL to 10.3±1.9 g/dL.[20]

CONCLUSIONS AND SUGGESTIONS

In this study, the mean increase in Hb levels was 1.76+0.78 g/dL at 6 hours after PRC transfusion and 1.9+0.78 g/dL at 24 hours after PRC transfusion in non-hematological malignancy patients. There was a difference in delta Hb levels 6 hours and 24 hours after PRC transfusion, respectively, compared with the baseline Hb levels in non-hematological malignancy patients, which was statistically significant but not clinically significant.

From the study results, it can be suggested that if the patient is assessed for further transfusion, it is better to evaluate the hemoglobin at 6 hours after the PRC transfusion. If the patient is judged not to need continuous transfusion, hemoglobin evaluation can be performed 6-24 hours after the PRC transfusion. Variations in the type and stage of malignancy and the intravascular oxidative conditions suffered by the patient may be limitations of this study.

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