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CD3+ and CD4+ T Lymphocyte Counts in Recently Transfused Patients with Sickle Cell Disease in Zaria, Nigeria.Saidu H.¹, Musa B.O.P.², Jamoh B.Y.³, Babadoko A.A.⁴, Ahmad A.E.⁵ and Awwalu S.⁴

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Abstract

Blood transfusion is the mainstay in the management of SCD and this may present with attendant risk of transfusion induced immunomodulation (TRIM). The aim of the research was to enumerate CD3+ and CD4+ T lymphocytes in recently transfused SCD patients in steady state. The objectives are to compare the CD3+ & CD4+ counts of test and control groups and to see the effects of time after transfusion and dose on the counts. One hundred participants with SCD were recruited in the study, 50 participants were transfused within the previous six months to sample collection and the remaining 50 had no history of blood transfusion within six months to time of sample collection. All the participants were HIV seronegative adults in steady state. CD3+ and CD4+ T lymphocyte count was done using BD FACS count flow cytometer. There was no significant difference between the study group and the control group in CD3+ and CD4+ T-lymphocyte count; mean and standard deviation: 2333.00±1069 cells (/μL) and 2429.0±847 cells (/μL) respectively, $p=0.6198$. There was also no significant correlation between number of blood units given and T cell subsets (CD3+ and CD4+ T cell count). Spearman's ρ and p values for CD4+ and CD3+ T cells = (-0.0552 and $p=0.7030$) and ($\rho= -0.0553$ and $p=0.7063$). Blood transfusion is not associated with changes in CD3+ and CD4+ T lymphocyte subset counts in patients with sickle cell disease.

Keywords: Sickle cell disease, Transfusion, CD3+ and CD4+ T cell subsets.

Introduction

Sickle cell disease (SCD) is a disorder arising from the inheritance from both parents of sickle haemoglobin (HbSS) or of HbS from one parent and another abnormal Hb variant (example HbC, HbD, Thalassaemia etc) from the other parent (Wang, 2004). This disorder leads to sickling of the red blood cells (RBCs) following deoxygenation. This initial trigger leads to a spectrum of presentations such as vaso-occlusive crises and end organ damage depending on disease severity. About 150,000 neonates are born with this disorder yearly in Nigeria (Akinyanju, 2008) and millions worldwide. SCD is predominant among people of Black African, Afro-Caribbean, Mediterranean, Middle East and Indian descent (Booth *et al.*, 2010).

Individuals with SCD have a chronic disease punctuated by exacerbations that require blood transfusions such as splenic sequestration, and transient red cell aplasia. Others include cerebral syndromes, acute chest syndromes and stubborn bone pains. Transfusion of red blood cells (RBCs) can be life-saving in these settings (Telen, 2001). However, transfusion carries risks, many of which are greater in individuals with SCD than in the general population. The approach to transfusion in SCD, as in other conditions, must balance benefits and risks, both in decisions regarding when to transfuse and in the technical aspect of how transfusions are administered (Clinical Use of Blood, 2008).

Infection is a significant contributor to morbidity and mortality in SCD. Functional asplenia or

autosplenectomy and attendant hyposplenism results in increased susceptibility to infection, especially to encapsulated bacterial pathogens (Booth *et al.*, 2010). Similarly, infections initiate a cascade of SCD-specific pathophysiological changes (Booth *et al.*, 2010). Inefficient opsonophagocytosis results from aberrations of the alternative complement pathway and the hypersplenism. Additionally, deficiency of specific circulating antibodies, impaired leucocyte function and reduced CD4⁺/CD8⁺ ratio despite an existing leucocytosis are some of the mechanisms that have been reported to account for the immunocompromised state in patients with SCD (Booth *et al.*, 2010).

Blood transfusion, a common modality of SCD treatment (Lal and Vichinsky, 2005), has been identified to lead to immunomodulation (Opelz, 1989). There is a dearth of specific data on the effect of recent blood transfusion on the proportions of CD4⁺ T cells among SCD patients. At best the few studies available give conflicting findings; some suggesting no effect (Wong, 1995) and yet still others suggesting a decrease (Kaplan, 1984) The aim of this study was to assess the alteration in the T cell compartment through CD3⁺ and CD4⁺ T cells enumeration in recently transfused patients with SCD.

Materials and Methods

Study design and participants

This is an analytical cross-sectional study, the study participants comprised of 50 steady state adult patients with SCD who were transfused (not more than six months to time of enrollment) who were HIV seronegative and attending the SCD clinic of Ahmadu Bello University Teaching Hospital, Shika – Zaria. A patient is said to be in a steady state when he/she has no history of acute painful episodes that requires admission into hospital emergency unit for at least four consecutive weeks (Ballas, 2010). Participants with SCD in steady state that have not been recently transfused were used as controls.

Sampling Technique

The sampling technique used was simple random sampling method.

Ethical Considerations

Approval from the Health Research Ethical Committee of ABUTH (ABUTH/HREC/R18/2015) and written informed consent from the participants was obtained prior to the commencement of samples collection.

Clinical data collection

An interviewer- administered questionnaire was used to collect information on patients' socio-demographic details and clinical history. Patients' folders were also accessed for current anthropometric and clinical details.

Sample processing and analysis for CD3⁺ and CD4⁺ cells

Five (5) mL of venous blood was taken from the participants and controls participating in the study. All the samples were analyzed within five hours of collection. Reagents for CD3⁺ and CD4⁺ cell count were obtained from BD Bioscience company 2350 Qume Drive, San Jose, CA 95131 USA (BD FACSCount™ reagent kit CD3/CD4 catalogue number: 340167 and BD FACSCount™ control kit catalogue number: 340166), the count was done using BD FACSCount instrument (San Jose, USA), manufacturer's instructions were strictly followed.

Data analysis

The data was edited and summarized using Microsoft excel 2013. Following normality test, continuous variables were summarized as mean and standard deviation and discrete variables were summarized using frequencies and percentages. Independent samples t test, ANOVA and Spearman's rank correlation (for ranked transfusion dose against immunocytes count) were used to test for significance. Tukey and Cramer's post hoc analysis was conducted for all significant ANOVA results and significance level was set at $p \leq 0.05$. Analysis was done using Graph pad Prism version 5.03

Results

There was no statistically significant difference in CD3⁺ and CD4⁺ T cell counts between subjects and controls ($p=0.4866$ and 0.6198) respectively. Similar findings were seen in the CD3⁺CD4⁺ T lymphocytes (CD8⁺ T cell) compartment and the CD4/CD8 ratio

(CD4+/CD3+CD4-T cell (/μL) (p=0.7957 and 0.5074) respectively (Table 1). In table 2, the participants were grouped as follows: those transfused 1-2 months ago, 3-4 months and 5-6 months ago. No statistically significant difference was observed in the CD3+ count, CD3+CD4⁻ T cell count and CD4/CD8 ratio for the three subsets (p=0.1475, 0.6936 and 0.6521) respectively. However, a significant difference was observed in the CD4+ T cell count

(p=0.0115) between the subsets. No significant relationship was found between CD3+ T cell and CD4+T cell counts and blood transfusion ρ and p values (-0.0553 and 0.6714) and (-0.07937 and 0.07033) respectively. Figure 1 and 2 show no significant correlation between number of blood units given and T cell subsets (CD3+ and CD4+ T cell count); Spearman's ρ and p values for CD4+ and CD3+ T cells = (-0.0552 and p=0.7030) and (ρ= -0.0553 and p=0.7063).

Table 1: Immunophenotypic parameters in SCD Subjects and Control groups

Parameter	Study group	Control group	t- ratio	p- value#
CD3+T (cell/μL)	2333.00± 1069	2429.0± 847	0.4977	0.6198
CD4+T (cell/μL)	1330.00± 599	1407.0± 503	0.6961	0.4880
CD3+CD4 ⁻ T (cell/μL)	1003.00± 644	1033.0± 503	0.2596	0.7957

Key: #determined using unpaired t-test, WBC: White Cell Count, CD: Cluster of Differentiation, CD3+CD4⁻T cell: Total T cells (CD3+) - CD4+ T cells

Table 2: Comparison of immunophenotypic parameters and time since last transfusion in the Study group

Parameter	Duration after transfusion in months			p value#
	1-2months (n=29)	3-4months (n=17)	5-6months (n=4)	
CD3+(cells/μL)	2584 ± 1055	1965 ± 1130	2082 ± 101	0.1475
CD4+(cells/μL)	1528 ± 612	995 ± 487	1313 ± 297	0.0115*
CD3+CD4 ⁻ (cells/μL)	1055 ± 750	971 ± 545	769 ± 409	0.6936

Key: #Determined by one-way ANOVA. *-Statistically significant difference.

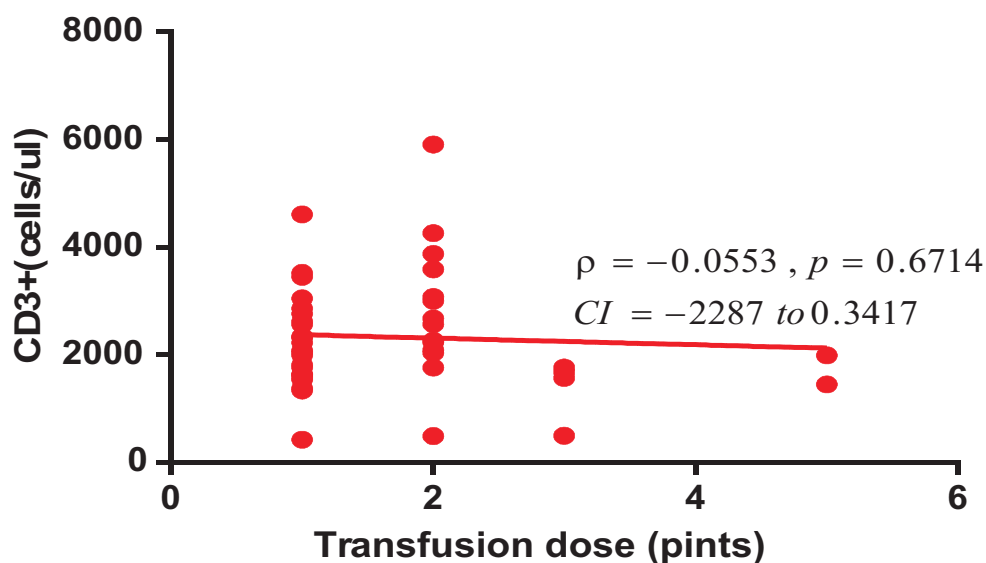


Figure 1: Transfusion dose and CD3+ T cell count. Scatter plot showing the relationship between CD3+ T cells and blood transfusion dose.

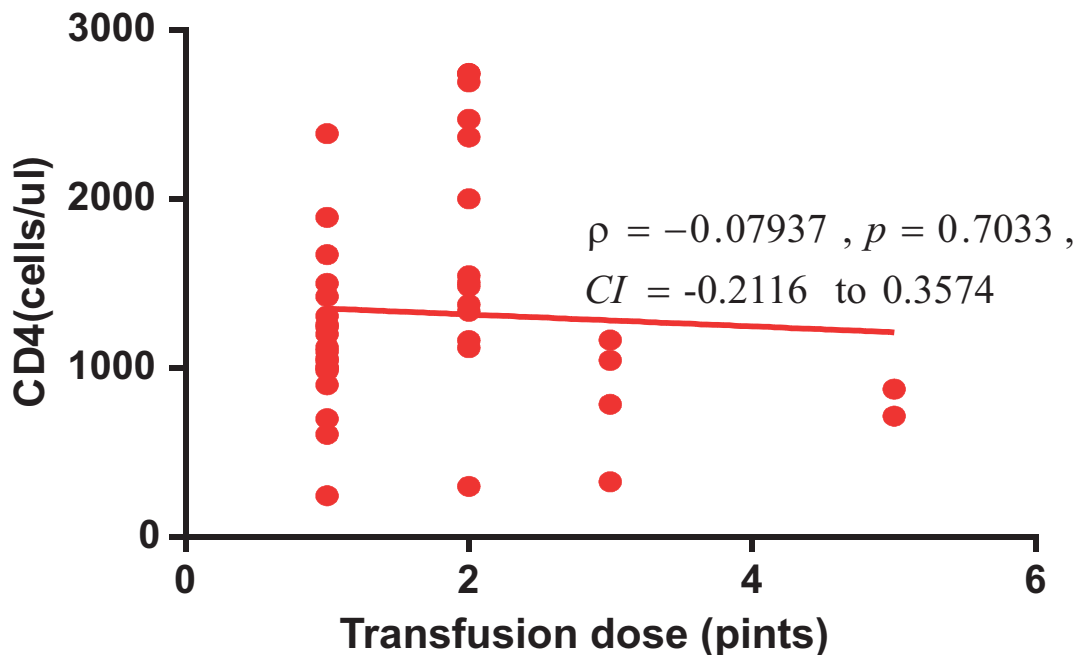


Figure 2: Transfusion dose and CD4+ T cell count. Scatter plot showing the relationship between CD4+ T cells and blood transfusion dose. (ρ and p values determine using Spearman correlation).

Discussion

White blood cells (WBCs) have important roles in the evolution of Transfusion -Related Immunomodulation (TRIM) although knowledge of the exact mechanisms is still evolving (Brown and Navarrete 2011). The absence of significant differences in CD3+ and CD4+ T cell counts between the subjects and the control groups is similar to the findings among SCD patients in other countries. Wong (1995) did not find any differences in lymphocyte subsets between transfused SCD patients and controls in the US. A similar observation was made by Nickel et al. (2013) in a study conducted in the US involving one hundred and eleven SCD participants (68 were patients with SCD on chronic transfusion, 26 on hydroxyurea and the remaining 17 were not on any treatment) and 29 normal healthy controls. Their findings revealed absence of significant alterations in T cell subsets attributable to blood transfusions except for the CD8+ T cell counts. CD4+ T cell subset analysis (central memory and effector memory) also yielded similar results.

Similarly, a more recent independent study using a different cohort of SCD patients in the US found that T cell subsets were not affected by

blood transfusion (Nickel, 2015). Purohit *et al.* (2012) made a similar observation in their study on immune response to viral infections in patients with sickle cell disease on chronic blood transfusion. This lack of relationship between transfusion and alterations in CD4+ T-Cell subsets has been described among patients with congenital clotting disorders. Thus, suggesting that the absence of such a response is not specific to SCD patients.

However, Kaplan (1984) reported contrary findings in their study on four groups of participants: 30 SCA patients on monthly transfusion, 30 untransfused SCA participants, 87 study group with haemophilia or von Willebrand disease and 30 normal healthy controls. Their data revealed that transfused SCA patients have elevated CD4+ T cell counts and CD8+ T cell counts compared to those not transfused. Kaplan might have accidentally dealt with a cohort of SCD patients that persistently express the immune activated phenotype. These patients quickly become alloimmunised against donor antigens thus producing a pool of antigen specific CD4+ and CD8+ T cells (Bao *et al.*, 2013).

The absence of alterations in our study and other studies may be as a result of a short-lived response in the participants. Hence measurements were made after the effects have subsided. A more likely alternative may be the presence of qualitative, rather than quantitative, defects in the lymphocyte responses. Hence the ability to proliferate in response to recall antigens may be unaltered but the ability to get activated and produce the necessary cytokines as a response to neo antigens is compromised. The concept of selective, and qualitative, defects following blood transfusion was suggested by Opelz (1989). He suggested that only the monocyte and cytotoxic T cells were affected particularly in their ability to destroy their target antigens.

Mouse models have supported the presence of functional defects following blood transfusions. Busch (2013) have demonstrated a reduction in IL-2 production by lymphocytes after transfusion. Additionally, a preponderance of Th2-type cytokines (IL-4, IL-5, IL-10) over Th1 cytokines (gamma interferon, IL-2) in post transfusion stimulated lymphocytes has been described in both mice and humans. The Th2 cytokine pattern is associated with decreased cytotoxic cell functions and inhibition of macrophage activation. This pattern potentially accounts for the increased tumor recurrence, increased infection rates, more rapid progression of viral diseases, and reduction in inflammatory bowel disease episodes in recipients of allogeneic transfusion (Shiba *et al.*, 2009).

Another plausible explanation could be that the immunomodulatory effect of the transfusion is masked by the leukocytosis inherently associated with SCD; the study group are likely to have higher frequency of SCD associated complications (VOC, ACS, anaemia etc) and hence the increased dependence on transfusion (Wang, 2004). These complications are associated with elevation of lymphocyte count and as such the blood transfusion may not easily counter this background leukocytosis (Wang, 2004).

Our study also revealed that the study group had marked reduction in CD4+ counts 3-4 months

after transfusion followed by a recovery. Anamnestic responses to recall allogeneic antigens from previous transfusions or sensitizations may be responsible for this (Shiba *et al.*, 2009). The absence of significant differences in the CD4+:CD3+CD4T cell (CD4:CD8 ratio) across the three strata of transfusion doses in our study is similar to the observations made by Purohit *et al.* (2012). However, Amy and Kenneth (2003) and Nickel *et al.* (2013) reported lower ratios in multiply-transfused SCD participants. In both reports however, the ratios were low due to increases in the CD8+ T cell counts and not decreases in the CD4+ counts. This increase in CD8+ cells may result from responses to viruses such as EBV, HBV and CMV (Kaplan *et al.*, 1984).

The variability in responses described in this and other studies may have genetic bases which were not accounted for in this study. Racial and inter-individual genetic differences have been shown to influence patterns of lymphocyte responses (Rubelt *et al.*, 2016; **Federico *et al.*, 2005**). It will be important for future studies to determine the genotype-phenotype interactions in lymphocyte responses to blood transfusion among SCD patients.

Conclusion

SCD patients have significant reduction in CD4+ T lymphocyte counts in the 3rd to 4th months following blood transfusion. This quantitative deficit is however abolished 5 to 6 months post blood transfusion.

Recommendations

- Measures such as component specific transfusion and leukodepletion should be put in place to minimize unnecessary exposure to unwanted donor antigens.
- Subsequent studies should among other things consider the functionality of T and B cells and perhaps other cells forming the interface between adaptive and innate immunity particularly the antigen presenting cells (APCs) among patients with SCD.

Conflict of interest

The authors declare no conflict of interest

Authors' contribution:

Saidu H conceived the idea and carried out the laboratory work, Musa BOP, Jamoh BY and Babadoko AA supervised the whole work including drafting of manuscript, Awwalu S, Ahmad A.E and Saidu H carried out the statistical analysis and discussed the result.

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