# Assessment of $CD4^{+}CD28^{null}$ T- Lymphocytes in Pediatric Patients with Sickle Cell Disease

Thesis

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# List of Abbreviations

| Abb. | Full term |
|---|---|
| *ACS* | *Acute chest syndrome* |
| *AVN* | *A vascular necrosis* |
| *FDA* | *Food and Drug Administration* |
| *Hb* | *Hemoglobin* |
| *HBB gene* | *Hemoglobin β- globin gene* |
| *HbF* | *Fetal hemoglobin* |
| *HbS* | *Sickle cell haemoglobin* |
| *HbSS* | *Hemoglobin SS* |
| *HPLC* | *High-performance liquid chromatorgraphy* |
| *HU* | *Hydroxyurea* |
| *IFN* | *Interferon gamma* |
| *IgM* | *Immunoglobulin M* |
| *NO* | *Nitric oxide* |
| *NSAIDs* | *Nonsteroidal anti-inflammatory agents* |
| *PAH* | *Pulmonary Hypertension* |
| *PS* | *Phosphatidylserine* |
| *RBCs* | *Red blood cells* |
| *ROS* | *Reactive oxygen species* |
| *SCA* | *Sickle cell anemia* |
| *SCD* | *Sickle cell disease* |
| *T lymphocytes* | *Thymus-derived lymphocytes* |
| *TCD* | *Transcranial Doppler* |
| *TRACP 5b* | *Tartrate-resistant acid phosphatase 5b* |
| *VCAM* | *Vascular cell-adhesion molecule* |
| *VOC* | *Vaso-occlusive crisis* |
| *VOE* | *Vaso-occlusive pain events* |
| *WBCs* | *White blood cells* |

# Abstract

**Background:** Sickle cell disease (SCD) is increasingly appreciated as an inflammatory condition associated with alterations in immune phenotype and function. $CD4^+$ T lymphocyte influence the functions of virtually all other cells of the immune system, including other T cells, B cells, macrophages and natural killer (NK) cells. $CD4^+CD28^{null}$ T-cells are a subset of long-lived directly cytotoxic $CD4^+$ T lymphocytes that have pro-inflammatory functions characterized by the production of high levels of interferon-gamma (IFN-gamma). **Aim:** To assess the percentage of $CD4^+CD28^{null}$ T cells as well as IFN-gamma levels in 40 children and adolescents with SCD compared with 40 age- and sex-matched healthy controls and evaluate their relation to hemolysis, iron overload, vaso-occlusive crisis (>3 attacks/year) and response to therapy. **Methods:** SCD patients in steady state were studied focusing on history of frequent vaso-occlusive crisis, transfusion history, hydroxyurea therapy, hematological profile and serum ferritin. Analysis of T cells was done by flow cytometry for assessment of $CD4^+$ T lymphocytes and $CD4^+CD28^{null}$ T-cells. Serum levels of interferon-gamma were assessed by enzyme linked immunosorbent assay (ELISA). **Results:** Patients with SCD had higher WBCs and lower hemoglobin level compared with controls. $CD4^+$ T lymphocytes, $CD4^+CD28^{null}$ T-cells and IFN-gamma levels were significantly higher in patients than in controls. Patients with history of frequent sickling crisis had higher percentage of $CD4^+CD28^{null}$ T-cells and IFN-gamma than those without. The levels of these cells and IFN-gamma were significantly lower among hydroxyurea-treated patients as well as those on combined chelation and hydroxyurea therapy. There were significant positive correlations between each of CD4+ T lymphocytes and $CD4^+CD28^{null}$ T-cells and transfusion index and iron overload per day. Moreover, $CD4^+CD28^{null}$ T-cells were positively correlated to IFN-gamma while negatively correlated to cardiac T2* and duration of hydroxyurea therapy. A significant positive correlation was found between IFN-gamma and $CD4^+CD28^{null}$ T-cells while it was negatively correlated to cardiac T2* and duration of hydroxyurea. **Conclusions:** Expression of $CD4^+CD28^{null}$ T-cells with increased production IFN-gamma highlights the role of immune dysfunction in pediatric patients with SCD. Alteration of this subset of T lymhocytes is related to increased iron overload and higher incidence of vaso-occlusive crisis. Hydroxyurea and/or iron chelation therapy significantly contributes to the normalization of cytotoxic lymphocytes and attenuates immune dysfunction.

# INTRODUCTION

Sickle cell anemia is a monogenic hemoglobinopathy wherein glutamic acid, the sixth amino acid in the β-globin chain, is displaced by valine. This results in hemoglobin polymerization and sickling morphology during hemoglobin desaturation. Clinically, the disease is characterized by chronic hemolysis, intermittent vaso-occlusive events, and organ injury ***(Belcher et al., 2014)***.

In sickle cell disease (SCD), hemoglobin molecules polymerize intracellularly and lead to a cascade of events resulting in decreased deformability and increased adhesion of red blood cells (RBCs). Decreased deformability and increased adhesion of sickle RBCs lead to blood vessel occlusion (vaso-occlusion) in SCD patients ***(Alapan et al., 2016)***.

SCD is a hypercoagulable state with chronic activation of coagulation and an increased incidence of thromboembolic events ***(Whelihan et al., 2016)***.

Patients with sickle cell anemia (HbSS), particularly children, have an increased susceptibility to infection leading to increased mortality. Opsonophagocytic defect due to an abnormality of the alternative complement pathway, deficiency of specific circulating antibodies, impaired leucocytes function and loss of both humoral and cell-mediated immunity are some of the other mechanisms that have been reported to account for

immunocompromised state in patients with sickle cell disease ***(Ojo et al., 2014).***

Thymus-derived (T) lymphocytes play an important role in cellular immunity. In the blood, T lymphocytes constitute 60-70% of peripheral lymphocytes ***(Hoffbrand et al., 2014)*** and are also found in the paracortical areas of lymph nodes and periarteriolar sheath of the spleen. About 60% of mature T cells express CD4 (Helper) and 30% express CD8 (Cytotoxic). By secreting cytokines, $CD4^+$ T lymphocyte influence the functions of virtually all other cells of the immune system, including other T cells, B cells, macrophages and natural killer cells ***(Ojo et al., 2014).***

Studies have shown that patients with SCD have elevated white blood cells (WBCs) counts, absolute neutrophil count, total lymphocytes, monocytes, total T cells and total B cells. Total CD4+ T cells were also increased and this increase in $CD4^+$ counts was more pronounced than SCD-associated increased in $CD8^+$ count, the increase in total $CD4^+$ T cell counts was driven by increases in all of the $CD4^+$ T cell subpopulations (CD4+ T-naive, T-memory and T-regulatory), and was accompanied by increased number of proliferating (Ki67+) $CD4^+$ T cells. In contrast, CD8+ T cells only demonstrated significant increase in memory (not naive) and proliferating T cells ***(Robert et al., 2015).***