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## Original Article

# Assessing the Difference Between the Number of CD3+20+ T Cells by Flowcytometry in Idiopathic Nephrotic Syndrome Patients in Relapse and Remission Period: A Pilot Study

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## ABSTRACT

**Background and Aim:** The pathogenesis of idiopathic nephrotic syndrome (INS) remains unclear. Previously, the INS was assumed a T cell-mediated disease. Cytokines secreted from T helper 2 cells, reduced T regulatories, which led to activation of T effector cells, resulting in the increased permeability of glomerulus and nephrotic syndrome. The remedial influence of B cell depletion by anti-cluster of differentiation marker 20 factors suggests a role of B cells in the pathogenesis of the disease. However, the exact mechanisms of action of these medications are unknown. One of these mechanisms is the effect on CD3-20+ T cells.

**Methods:** A total of 10 patients in relapse of INS and 10 patients in remission were included in this study. The number of CD3-20+ T cells was calculated by flow cytometry in these two groups.

**Results:** In the relapse group, on average, 1.10% of T cells were CD3-20+ cells; in the remission group, the same data was 0.41%.

**Conclusion:** The mean number of CD3-20+ T cells in the remission group was lower than in the relapse group; however, there was no statistical difference between the two groups.

**Keywords:** T-lymphocytes, B-lymphocytes, Cluster of differentiation markers (CD20 and CD3), Flowcytometry

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## Introduction

Nephrotic syndrome is among the most common chronic kidney diseases during childhood. It is defined by the presence of massive proteinuria, hypoalbuminemia, peripheral edema, and hyperlipidemia. Although nephrotic syndrome may be secondary to many renal diseases, idiopathic nephrotic syndrome (INS) is the most frequent cause in childhood. Secondary causes include infections, malignancies, genetic disease, drugs and toxins, primary and secondary glomerulonephritis, and so on [1].

Proteinuria is secondary to a defect of the glomerular filtration barrier. The glomerular filter has three layers: Fenestrated endothelium, glomerular basement membrane, and podocyte. Podocytes are highly specialized cells wrapped around capillaries. Podocyte foot process effacement is the first morphological change that even without the loss of an entire cell, leads to massive proteinuria. Actin cytoskeleton change is the key point of foot process effacement. Albumin in the space of the bowman's capsule leads to hyperfiltration and increases stress damage to podocytes. Following the loss of podocytes, more pressure is applied to other cells and they undergo hypertrophy. The capacity of hypertrophy in podocytes is limited. Hypertrophied cells cannot maintain the normal structure of the foot process, as a result, podocytes are more likely to be torn off [2].

Although the pathogenesis of INS is unknown, a defect in the immune system is the cause. Given that the disease starts with an underlying infectious agent, allergy, etc. responses to treatment with immunosuppressive drugs can confirm the role of the immune system. Different parts of the immune system have been investigated in the pathogenesis of the disease.

The spontaneous remission of disease after measles infection, absence of immune complex deposition, and the relationship between nephrotic syndrome and T cell lymphoma support the role of cellular immunity in the pathogenesis of INS.

Cluster of differentiation 4+helper T cells (Th) can be differentiated into two distinct subsets, namely Th1 and Th2. Interleukin (IL) 13, a cytokine of Th2, increased during relapse. IL-13 arranges the switching of immunoglobulin production toward immunoglobulin E, which is increased in some cases of minimal change nephrotic syndrome. Meanwhile, the increase in IL-13 expression is accompanied by a decrease in nephrin and podocin and vice versa and an increase in CD80.

Numerous laboratory and human studies show that following a triggering event, the number of T regulatory cells decreases and proteinuria occurs. Also, in 20% of patients with immune dysregulation, polyendocrinopathy, enteropathy X-linked syndrome, which is an inherited immunodeficiency and severe Treg deficiency, nephrotic syndrome and minimal change nephrotic syndrome occur.

Other important cells that originate from CD4+ progenitors are Th17. Th17 and Treg have the opposite effect, high Th17/Treg shows the maintenance of inflammation. An excessive increase in the number of Th17 cells has been observed in the peripheral blood of INS patients. Additionally, the increase of these cells is related to resistance to corticosteroid treatment. Determining the profile of each person's immune cells, including the Th17/Treg ratio can help choose the treatment [3].

Focal segmental glomerulosclerosis recurrence after transplantation and the effect of plasmapheresis in its treatment, occurrence of nephrotic syndrome in neonates of mothers with focal segmental glomerulosclerosis, albuminuria in mice after plasma injection of patients with focal segmental glomerulosclerosis are indicators of the role of circulatory factor in pathogenesis of INS [4]. Several circulating factors have been identified to date as follows.

Soluble urokinase plasminogen activator receptor (SuPAR) binds to and activates αvβ3-integrin of podocytes. It changes in the mobility of podocytes and the destruction of the podocyte foot process led to proteinuria.

Cardiotrophin-like cytokine factor 1 (CLCF-1) is a component of the IL-6 family. It decreases nephrin in podocytes. The level of CLCF-1 in patients with recurrent focal segmental glomerulosclerosis is nearly 100 times more than in the healthy group [5].

Hemopexin is a plasma β-1 glycoprotein. It binds heme and is released into serum thus playing a role in iron homeostasis. This molecule has also serine protease activity. It induces reorganization of the actin in the cytoskeleton of podocytes and lessens the glycocalyx [6].

The presence of anti-CD40 antibodies in the serum of patients with recurrent focal segmental glomerulosclerosis shows a probable effect of B cells in the pathogenesis of the disease. CD40, a member of the tumor necrosis factor receptor superfamily, mediates immune activation and regulates tumor apoptosis. CD40 can present on damaged podocytes. anti-CD40 antibodies prolong the suPAR-mediated hot up of integrins which increases podocyte mobility and their loss [7].

With the continued improvement of proteinuria after treatment with rituximab, attention was drawn to the effect of B cells in the pathogenesis of nephrotic syndrome. The association of Hodgkin and non-Hodgkin's lymphoma or Epstein-Barr virus infection, which mainly targets B cells, with nephrotic syndrome, is another sign of the effect of these cells. Extracorporeal immunoadsorption methods also cause remission in patients. It indicates that the factor that is the cause of proteinuria is either an immunoglobulin or it can bind to immunoglobulin [4].

B cells can make pathogenic antibodies against podocyte components, such as anti-CD40 antibodies, anti-ubiquitin carboxy-terminal hydrolase L1 antibodies, and anti-nephrin antibodies.

A recent study presents that hyposialylated immunoglobulin M works against T cells in a group of steroid-sensitive nephrotic syndrome patients. Sialylated immunoglobulin M targeting T cells, is quickly internalized and restrains T-cell activation while hyposialylated immunoglobulin M remains on the cell surface and fails to do inhibitory roles. Also, the binding of hyposialylated immunoglobulin M leads to T cell's unresponsiveness to steroid prohibition.

B cells are capable of secreting both IL-13 and tumor necrosis factor α, as well as other cytokines such as IL-4, interferon-gamma, IL-6, and IL-17.

Proteinuria occurrence in lipopolysaccharide-injected severe combined immunodeficient mice, which are depleted of T- and B-cells, support the podocyte roles. Under inflammatory conditions, podocytes increased expression of major histocompatibility class II molecules. Podocytes may play a role as antigen-presenting cells. Podocytes also present CD80, a molecule needed for T-cell activation. CD80 cooperates with CD28 on CD4+ T-cells, mediating their activation into Teff cells. Expression of glomerular CD80 was seen in renal biopsies of focal segmental glomerulosclerosis patients. Therapy with abatacept, a molecule that inhibits CD80, can induce recovery in post-transplant relapse of focal segmental glomerulosclerosis.

A small number of human CD3+ T cells in peripheral blood, to a small extent express CD20 on their surface. The population of these cells in healthy people has been reported differently in various studies. For example, a study on 142 persons, constitutes an average of 1.6% of the total CD3+ cells (0.1%-6.8%), and their absolute number is about 28 cells per microliter. The source of CD20+ T cells is not almost clear. CD20 protein expression is not present in T cells of human cord blood, suggesting that this molecule was acquired thereafter in development. CD20+ T cells are identified via flow cytometry as $CD3+CD19^{-}$ cells with dim expression of CD20. They may present CD4, CD8, or none of them; however, they are particularly enriched with CD8 compared to CD20- T cells. Compared to CD20- T cells, CD20+ T cells also express significantly more IL-2, IL-4, IL-10, IL-17, tumor necrosis factor, interferon-gamma, and granulocyte-macrophage colony-stimulating factor as assessed by flow cytometry [5].

The most common disease in which the role of these cells has been investigated is multiple sclerosis. CD20+ T cells express more binding molecules and chemokine receptors than CD20- T cells, thus the capability of moving to the central nervous system is higher in this cell population.

Current treatment methods, such as fingolimod, dimethylformamide, natalizumab, and alemtuzumab are effective on the CD20+ T cell population [8].

## Materials and Methods

In this pilot study, patients with INS confirmed by the diagnostic criteria of their disease, either newly diagnosed or have frequent relapses or steroid dependency were included.

The data were analyzed using the SPSS software, version 20, and the values of Mean±SD were determined.

Patients who had two or more relapses in the first 6 months after stopping primitive therapy, or ≥3 relapses in 1 year defined as frequent relapse nephrotic syndrome and who had two seriate relapses when on alternate day steroid or within 14 days of stopping therapy defined as steroid-dependent nephrotic syndrome. Either steroid-dependent nephrotic syndrome or frequently relapsing nephrotic syndrome are included in the study. Patients may also have used other steroid-sparing medications.

Relapse is defined as urine dipstick protein ≥3+ for 3 seriate days, urine to protein creatinine ratio ≥2 mg/mg, or urine protein ≥40 $mg/m^2/h$. Patients in remission have dipstick protein nil or trace for 3 seriate days, urine protein creatinine ratio ≤0.2 mg/mg, or urine protein ≤4 $mg/m^2/h$.

The number of CD20+ T cells is measured by flow cytometry at the time of disease relapse and the same measurement is performed in another group of patients who are in remission.

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The exclusion criteria were steroid-dependent nephrotic syndrome, congenital cases of the disease, and genetic and syndromic types.

In addition to CD20+ T cells, the population of CD4+ T and CD8+ T cells were also compared at the time of relapse and recovery. It was also determined that the majority of CD20+ T cells are CD4+ or CD8+.

## Results

The mean age of patients at the time of relapse was 7.9±3.07 years and at the time of remission was 9.0±3.09 years (Figure 1).

In the relapse and remission group, two patients were female and the other 8 were male. The lowest age in the male group was 3 years and the highest was 13 years, while the females were between 7 and 12 years old.

Only 6 patients underwent kidney biopsy, two from the relapse group and four from the recovery group.

The mean number of CD20+ T cells in the relapse group was 1.10±1.2% and in the remission group was 0.41±0.25% (Figure 2).

Among these cells at the time of relapse, the number of CD4+ T cells was about 0.34% and CD8+ T cells was about 1.03% and at the time of recovery 0.7% and 0.23% were reported, respectively (Figures 3 and 4).

## Discussion

To the best of our knowledge, a similar study has not been conducted yet on the role of CD20+ T cells in the pathogenesis of INS in children; however, extensive research has been done on these cells in various diseases such as multiple sclerosis.

In the study of Webendörfer et al., which was conducted on a 70-year-old man with nephrotic syndrome, the presence and influence of CD20+ T cells in the pathogenesis of INS were discussed. This patient needed to repeat treatment with corticosteroid, but due to severe osteoporosis was treated with rituximab and made a complete recovery. The patient received a repeated dose of rituximab while he had no B cells, at this time the population of CD20+ T cells was evaluated, which disappeared after rituximab [9].

In the current study, this was conducted in a pilot form on 10 patients with nephrotic syndrome at the time of relapse and 10 other patients at the time of recovery. The number of CD20+, CD4+, and CD8+ T cells was measured by flow cytometry. The number of CD20+ T cells decreased during recovery compared to the time of relapse; however, there was no statistically significant difference. In the remission group, the predominant population of CD20+ T cells had CD8.

In the relapse group, the number of CD4+ T cells was higher and the number of CD8+ T cells was lower than remission group but none of them had significant statistical value.

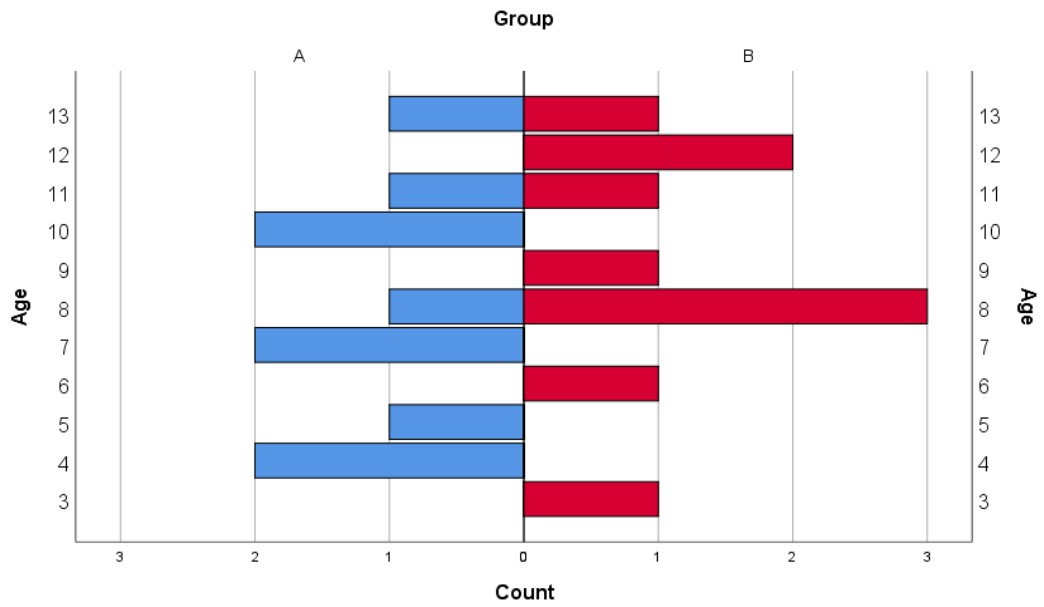


**Figure 1.** Age distribution in two groups (blue: Relapse and red: Recovery)

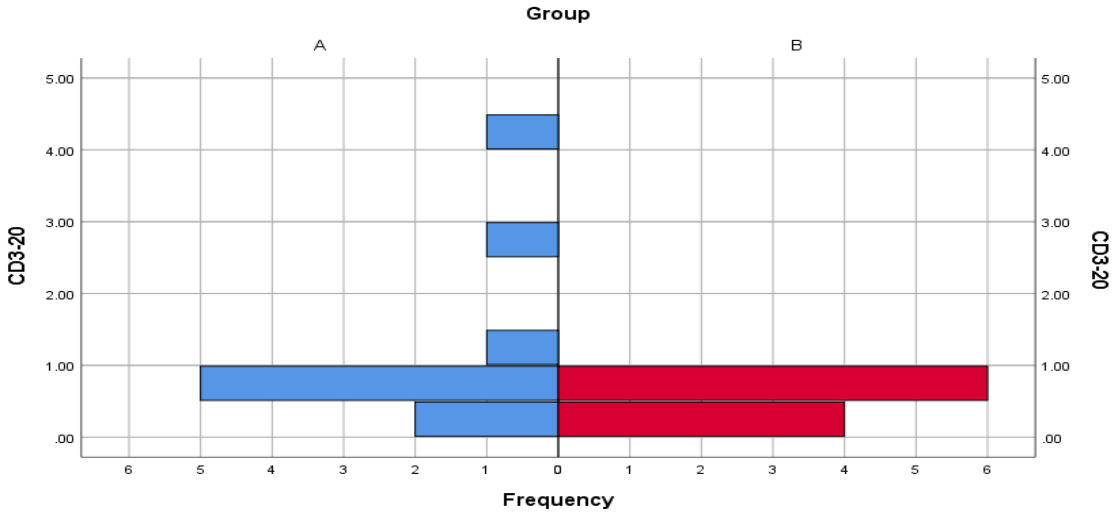


**Figure 2.** The number of CD20+ T cells in two groups (blue: Relapse and red: Recovery)

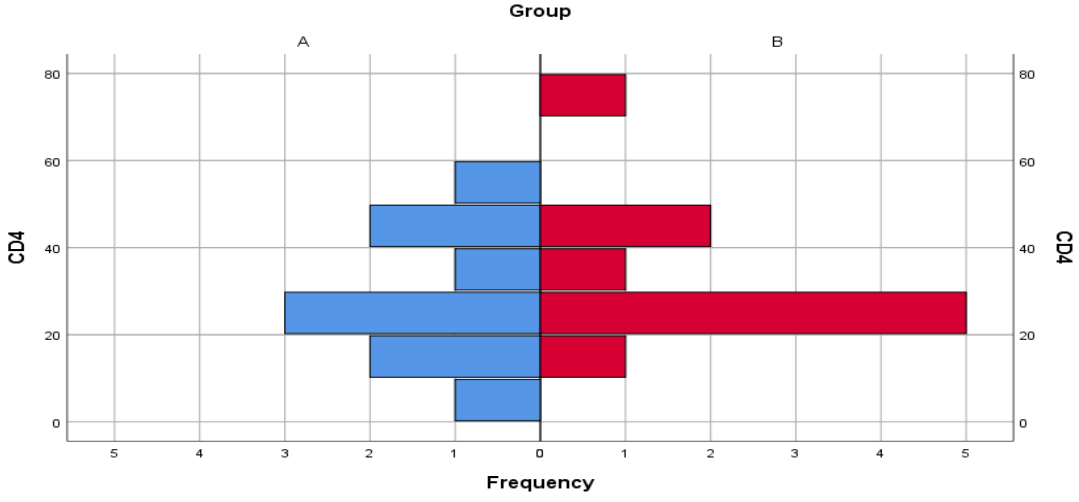


**Figure 3.** The number of CD4+ T cells in two groups (blue: Relapse and Red: recovery)

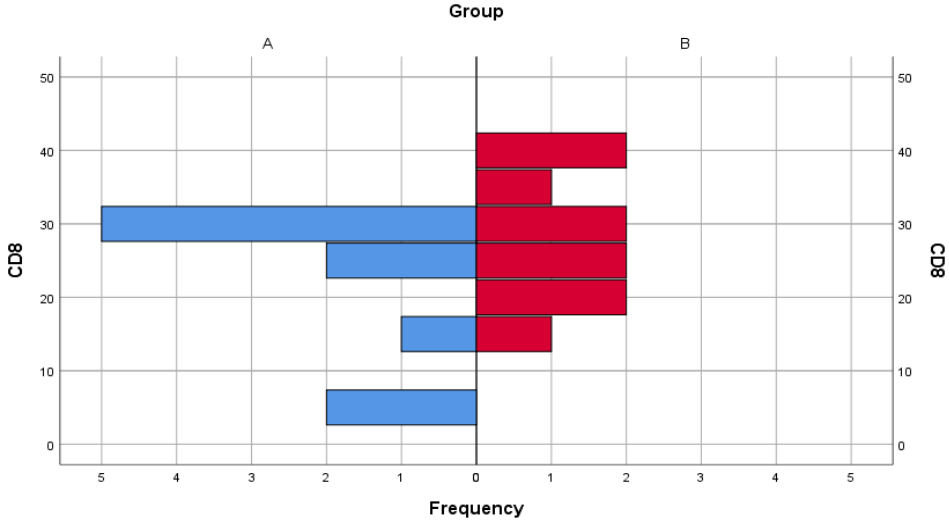


**Figure 4.** The number of CD8+ T cells in two groups (blue: Relapse and red: Recovery)

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## Conclusion

The mean number of CD3-20+ T cells in the remission group was lower than in the relapse group; however, there was no statistical difference between the two groups.

### Limitations

Considering that the study was conducted in a pilot method, the number of patients was small. If each patient was considered as a control at the time of remission, the number of intervening factors in the study would be less, and perhaps more comprehensive results could be obtained.

More extensive studies with a larger sample size are needed and it is suggested to select the control group more carefully to determine the role of CD20+ T cells in the pathogenesis of INS in children.

## Ethical Considerations

### Compliance with ethical guidelines

There were no ethical considerations to be considered in this research.

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### Authors' contributions

All authors equally contributed to the preparation of this article.

### Conflict of interest

The authors declared no conflict of interest.

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