PREDICTION OF RESPONSE TO STEROIDS IN PEDIATRIC IDIOPATHIC NEPHROTIC SYNDROME

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ABSTRACT

Background: Nephrotic syndrome (NS) is a clinical manifestation of glomerular diseases characterized by heavy (nephrotic-range) proteinuria, hypoalbuminemia, edema, and hyperlipidemia. Nephrotic syndrome is the commonest cause of chronic kidney disease in children, and is more common in children aged over 5 years. About 90% of cases of nephrotic syndrome in children are idiopathic and steroid sensitive, with better prognosis, while the remaining 10% of cases do not respond to steroids within 4 weeks of steroid therapy and are defined as steroid resistant nephrotic syndrome (SRNS). The most common cause of nephrotic syndrome in children is idiopathic nephrotic syndrome (INS) also known primary nephrotic syndrome (1). Recent studies have shown the participation of B lymphocytes in the INS, either directly by histological and hematological analysis by increasing in the number of these cells in the active phase of the disease or indirectly by the therapeutic effect of rituximab in reducing proteinuria and the number of B lymphocytes in peripheral blood.

Key words: Idiopathic Nephrotic Syndrome (INS). Steroid Response

I. NEPHROTIC SYNDROME:

Definition:
Nephrotic syndrome is defined as heavy proteinuria (>50 mg/kg/day or >40 mg/m2/hour determined quantitatively on an overnight collection of urine), accompanied by hypoalbuminemia (≤ 2.5 g/dl) or by spot urinary protein to creatinine ratio higher than 200 mg/mmmol or > 2 mg protein / mg creatinine. The triad of clinical findings associated with nephrotic syndrome arising from the large urinary losses of protein are hypoalbuminemia (≤2.5 g/dL), edema, and hyperlipidemia (cholesterol >200 mg/dL) (1).

Nephrotic syndrome is a group of clinical findings that is the result of massive renal losses of protein. Thus nephrotic syndrome is not a disease itself, but a manifestation of many different glomerular diseases. These diseases might be acute and transient, such as postinfectious glomerulonephritis, or chronic and progressive, such as focal segmental glomerulosclerosis (FSGS) other diseases might be relapsing and remitting, such as minimal change nephrotic syndrome (MCNS) (2).

Epidemiology:
Incidence
The incidence of idiopathic nephrotic syndrome (INS) varies among countries, Asia reporting a higher incidence in comparison to Western countries (3).

The incidence of minimal change disease is higher in children with a reported incidence of 2 per 100,000 per year in Caucasian children and higher rates in Arabian and Asian children. The average incidence of nephrotic syndrome is 2-16.9 per 100,000 children worldwide (3).
More than 95% of children with minimal change Nephrotic syndrome respond to corticosteroid therapy. The percentage of steroid responsiveness in mesangial proliferative disease is 50% and that with focal segmental glomerulosclerosis is 20% (4).

Thirty percent to 40% of steroid-resistant cases progress to end-stage renal disease within 5 years of disease process. Mesangial proliferative in 50%, membranoproliferative glomerulonephritis in 20–30%, focal segmental glomerulosclerosis in 21% of cases and membranous nephropathy in 7.3% progress to end-stage renal disease within 5 years of disease (5).

Clinical Presentation

History

Edema is the presenting symptom in about 95% of children with nephrotic syndrome. Early on, the edema is intermittent and insidious, and its presence may not be appreciated the child commonly presented by periorbital edema, which is ascribed to "allergies" until the edema progresses (6).

Edema usually appears first in areas of low tissue resistance (eg, the periorbital, scrotal, and labial regions). It can progress rapidly or slowly (1).

Edema became generalized and can be massive (anasarca). The edema is pitting and typically dependent in nature, being more noticeable in the face in the morning and predominantly in lower extremities later in the day (6).

A history of a respiratory tract infection immediately preceding the onset of nephrotic syndrome is frequent, but the relevance to causation is uncertain. Upper respiratory infections, otitis media, and other infections are often associated with relapses of idiopathic nephrotic syndrome (INS). Approximately 30% of children have a history of allergy. A hypersensitivity event, such as a reaction to bee sting or poison has been reported to precede the onset of INS in some cases. Anorexia, irritability, fatigue, abdominal discomfort, and diarrhea are common. GI distress can be caused by ascites, bowel wall edema, or both. Respiratory distress can occur, due to either massive ascites and thoracic compression or frank pulmonary edema, effusions, or both (6).

Physical Examination

The most common clinical finding is edema. The edema is pitting and is typically found in the lower extremities, face and periorbital regions, scrotum or labia, and abdomen (ascites). In those children with marked ascites. Mechanical restriction to breathing may be present, and the child may manifest compensatory tachypnea. Pulmonary edema and effusions can also cause respiratory distress. Hypertension can be present and is more common in children with FSGS and MPGN rather than MCNS. Physical findings can also due to complications of INS. Abdominal tenderness might indicate peritonitis. Hypotension and signs of shock can be present in children presenting with sepsis (7).

Thrombosis can cause various findings, including tachypnea and respiratory distress (pulmonary thrombosis/embolism), hematuria (renal vein thrombosis), and seizure (cerebral thrombosis) (8).

B-cells

B cells, also known as B lymphocytes, are a type of white blood cell of the lymphocyte subtype. They function in the humoral immunity component of the adaptive immune system by secreting antibodies.

Additionally, B-cells present antigen (they are also classified as professional antigen-presenting cells (APCs) and secrete cytokines. (9).

In mammals, B cells mature in the bone marrow, which is at the core of most bones. In birds, B cells mature in the bursa of Fabricius, a lymphoid organ. (The "B" from B cells comes from the name of this organ (9).

B cells, unlike the other two classes of lymphocytes, T cells and natural killer cells, express B cell receptors (BCRs) on their cell membrane BCRs allow the B cell to bind to a specific antigen, against which it will initiate an antibody response. (10).
B Cells Production and Maturation

Lymphoid tissues

- **Primary** – Bone marrow – Thymus
- **Secondary** – Lymph nodes – Spleen – Tonsils – Lymphoid tissue within GI and respiratory tracts

Like T cells, B cells are formed from multipotent hematopoietic stem cells (HSCs) in the bone marrow and follow a pathway through lymphoid stem cell and lymphoblast (12).

Unlike T cells, lymphoblasts destined to become B cells do not leave the bone marrow and travel to the thymus for maturation. B cells continue to mature in the bone marrow (9).

The first step of B cell maturation is an assessment of the functionality of their antigen-binding receptors. This occurs through positive selection for B cells with normal functional receptors. (10).

A mechanism of negative selection is then used to eliminate self-reacting B cells and minimize the risk of autoimmunity. Negative selection of self-reacting B cells can involve elimination by apoptosis, editing, or modification of the receptors so they are no longer self-reactive, or induction of anergy in the B cell. (10).

Immature B cells that pass the selection in the bone marrow then travel to the spleen for their final stages of maturation. There they become naïve mature B cells, (mature B cells that have not yet been activated) (12).

Mature B cell exit bone marrow, migrate to secondary lymphoid organs, then express both surface IgM and IgD as well as other molecules that mediate cell-cell and cell-ECM adhesive interactions (13).

Mature resting B cells express HLA-DR, CD19, CD20, and CD40. Also can recirculate between blood and lymphoid organs, entering B cell follicles in lymph nodes and spleen, responding to antigen encounter with T cell help, leading to antibody production (13).

After encountering their antigen in the extrafollicular regions of the lymphoid organs, become activated B cells and migrate to the follicular regions. From here, they exit to differentiate into memory B cells, late plasmablasts and plasma cells (14).

Specific markers, such as CD20, CD27, BAFF-R (B-cell-activating factor receptor), CD38 and CD138, identify the transitional phases of B cells from stem cells to plasma cells (13).

**B cell Activation**

Following maturation in the bone marrow and spleen, B cells remain in peripheral tissues until they encounter an antigen and are activated. B cell activation requires two distinct signals, and results in B cell differentiation into memory B cells or plasma cells. The first activation signal occurs upon antigen binding to B cell receptors (BCRs) (11).

Upon binding to the BCR, the antigen is internalized by receptor-mediated endocytosis, digested, and complexed with MHC II molecules on the B cell surface. (11).

The second activation signal occurs via either a thymus-dependent (Tcell-dependent) or a thymus-independent mechanism (Tcell-independent) (10).

Most B cell responses to antigen require the interaction of B cells with T helper cells (thymus-dependent activation).

Presentation of an antigen-class II MHC complex on a B cell enables it to act as an antigen-presenting cell (APC) to T cells (11).
T cell receptors (TCR) on T helper cells bind to the antigen-complexed class II MHC molecule on the B cell surface resulting in T cell activation. The activated T cell then provides a second activation signal to the B cell, which can occur through a variety of proteins (10).

Alternatively, there are a few types of antigens that can directly provide the second B cell activation signal (thymus-independent activation). These antigens include components of some bacterial cell wall components (e.g., lipopolysaccharide) or antigens containing highly repetitious molecules (e.g., bacterial flagellin) (11).

Upon activation, B cells proliferate and form germinal centers where they differentiate into memory B cells or plasma cells (14).

Following differentiation into plasma cells, additional signals initiate plasma cell antibody class switching and regulate antibody secretion. The primary function of plasma cells is the secretion of B cell clone-specific antibodies (14).

Each plasma cell secretes antibodies containing a clonally-unique antigen-binding region joined to a constant immunoglobulin (Ig) isotype-defining region (14).

Subtypes of conventional B cells

Conventional B cells, also referred to as B-2 cells, terminally differentiate into one of two common subtypes upon activation:

**Plasma B cells**: a plasma cell is the sentry of the immune system. The naïve B cell circulates throughout the body. When it encounters a unique antigen, the plasma cell takes in the antigen through receptor-mediated endocytosis (14).

Antigenic particles are transferred to the cell surface, loaded onto MHC II molecules and presented to a helper T cell. The binding of the helper T cell to the MHC II-antigen complex activates the B cell. The activated B cell goes through a period of rapid proliferation and somatic hypermutation (15).

Selection occurs for those cells that produce antibodies with a high affinity for that particular antigen. Once terminally differentiated, the plasma B cell only secretes antibodies specific for that antigen and can no longer generate antibodies to other antigens (15).

**Memory B cells**: memory cells are held in reserve, in the germinal centers of the lymphatic system, for when the immune system re-encounters a specific antigen (15).

During any repeat exposure the follicular helper T cell causes the memory cell to differentiate into a plasma B cell that has a greater sensitivity to that specific antigen. This jump-starts the immune system to mount a quicker, more powerful response than was possible previously (14).

**Other B cell subtypes include:**

**B-1 cells**: a minor subtype, only about 5% in humans, of self-renewing fetal B cells that act in a similar fashion to plasma cells. B-1 cells are primarily present during fetal and neonatal life (11).

**Marginal zone (MZ) B cells**: mature memory B cells that are found only in the marginal zone of the spleen. These cells can be activated through toll-like receptor-ligation and not necessarily through the B cell receptor (16).

**Follicular (FO) B cells**: these are mature, but inactive, B cells. This subset of B cells is primarily found in the follicles of the spleen and lymph nodes. Activation of these cells requires the aid of T cells. FO B cells can differentiate into either plasma or memory B cells (11).

**Regulatory B (Breg) cells**: Breg cells negatively regulate the strength of the immune response and inflammation by secreting chemical messages called cytokines, such as IL-10. Although these cells make up a small portion of
the B cell population (~0.5% in humans), it is thought that loss of functional Breg cells contributes to autoimmune disorders (9).

B cell-related pathology

Autoimmune disease can result from abnormal B cell recognition of self-antigens followed by the production of autoantibodies (9).

Autoimmune diseases where disease activity is correlated with B cell activity include scleroderma, multiple sclerosis, systemic lupus erythematosus, type 1 diabetes, and rheumatoid arthritis (9).

Malignant transformation of B cells and their precursors can cause a host of cancers, including chronic lymphocytic leukemia (CLL), acute lymphoblastic leukemia (ALL), hairy cell leukemia, follicular lymphoma, non-Hodgkin's lymphoma, Hodgkin's lymphoma, and plasma cell malignancies such as multiple myeloma, and certain forms of amyloidosis (9).

Therapeutic B cell depletion

Indications: Autoimmune disorders or B-cell malignancies

Rituximab

- Anti-CD20 monoclonal antibody
- Depletes B cells
  - Fc receptor gamma-mediated antibody-dependent cytotoxicity
  - Antibody-dependent complement-mediated cell lysis
  - Growth arrest
  - B cell apoptosis
  - Does not significantly alter immunoglobulin levels because long-lived plasma cells are CD20- (12).

Clusters of differentiation (CD).

Cluster of Differentiation (CD) are defined when a surface molecule found on some members of a standard panel of human cells reacts with at least one novel antibody (17).

CD molecules are defined or classified by the reference monoclonal antibodies to which they bind. There are many clusters of differentiation; many cells express more than one CD marker. A phenotypic characterization of a cell of hematopoietic origin may be made by examining the pattern of CD markers expressed at any given time. The physiologic function has been identified for many, but not all (19).

In terms of physiology, CD molecules can act in numerous ways, often acting as receptoror ligands important to the cell. A signal cascade is usually initiated, altering the behavior of the cell. Some CD proteins do not play a role in cell signaling, but have other functions, such as cell adhesion. CD for humans is numbered up to 371 (17).

While CD molecules are very useful in defining leukocytes, they are not merely markers on the cell surface. Most of them have an important function. In the example of CD4 & CD8, these molecules are critical in antigen recognition. Others (e.g., CD135) act as cell surface receptors for growth factors (17).

As both B- and T-lymphocytes mature, they go through several stages of development. During these stages, lymphocytes will acquire new CD markers, while other CD markers will diminish in their expression or be lost. Also associated with the TcR is a complex of proteins known as CD3, which participate in the transduction of an intracellular signal following TcR binding to its cognate MHC/antigen complex (18).
T-cells consist of a variety of subpopulations, each of which can carry out one or more specific immune functions. One important way in which these subpopulations can be distinguished is by the use of the two cell surface markers CD4 and CD8 (17).

**CD19**

B-lymphocyte antigen CD19, also known as CD19 molecule (Cluster of Differentiation 19), B-Lymphocyte Surface Antigen B4, T-Cell Surface Antigen Leu-12 and CVID3 is a transmembrane protein that in humans is encoded by the gene CD19 (19).

In humans, CD19 is expressed in all B lineage cells, except for plasma cells, and in follicular dendritic cells. CD19 plays two major roles in human B cells. It acts as an adaptor protein to recruit cytoplasmic signaling proteins to the membrane and it works within the CD19/CD21 complex to decrease the threshold for B cell receptor signaling pathways. Due to its presence on all B cells, it is a biomarker for B lymphocyte development, lymphoma diagnosis and can be utilized as a target for leukemia immunotherapies (19).

CD19 is widely expressed during all phases of B cell development until terminal differentiation into plasma cells. During B cell lymphopoiesis, CD19 surface expression starts during immunoglobulin (Ig) gene rearrangement, which coincides during B lineage commitment from hematopoietic stem cell. Throughout development, the surface density of CD19 is highly regulated. CD19 expression in mature B cells is three fold higher than that in immature B cells (17).

**Fig. (1): Human CD19 expression by hematopoietic cells within the spleen and lymphoid tissues.**

Figure (1) CD19 is expressed at high levels by all mature and activated (act) B cells, plasmablasts (blast), and some pre-B cells and plasma cells (PC) within the bone marrow and spleen, including B cells located within the marginal and mantle zones, and germinal centers of lymphoid tissues. Among hematopoietic and non-hematopoietic cells, CD19 is only expressed by B cells, not T cells or natural killer (NK) cells, monocytes/macrophages (M), neutrophils (N), red blood cells (RBC), or platelets (P). Although not independently confirmed, follicular dendritic cells are reported to express CD19 (19).

CD19 is expressed on all normal, mitogen-stimulated, and malignant B cells, excluding plasma cells. CD19 expression is even maintained in B lineage cells that undergo neoplastic transformation. Because of its ubiquity on all B cells, it can function as a B cell marker and a target for immunotherapies targeting neoplastic lymphocytes (17).

Decisions to live, proliferate, differentiate, or die are continuously being made during B cell development these decisions are tightly regulated through BCR interactions and signaling. The presence of a functional BCR is necessary during antigen-dependent differentiation and for continued survival in the peripheral immune system. Essential to the functionality of a BCR is the presence of CD19, CD19 is essential for B cell differentiative events including the formation of B-1, germinal center, and marginal zone (MZ) B cells (19).

Analysis of mixed bone marrow chimeras suggest that prior to an initial antigen encounter, CD19 promotes the survival of naive recirculating B cells and increases the in vivo life span of B cells in the peripheral B cell.
compartment. Ultimately, CD19 expression is integral to the propagation of BCR-induced survival signals and the maintenance of homeostasis through tonic signaling (17).

On the cell surface, CD19 is the dominant signaling component of a multimolecular complex including CD21, a complement receptor, CD81, a tetraspanin membrane protein (TAPA-1), and CD225. The CD19/CD21 complex arises from C3d binding to CD21; however, CD19 does not require CD21 for signal transduction. CD81, attached to CD19, is a part of the tetraspanin web, acts as a chaperone protein, and provides docking sites for molecules in various different signal transduction pathways (17).

Mutations in CD19 are associated with severe immunodeficiency syndromes characterized by diminished antibody production. Additionally, mutations in CD21 and CD81 can also underlie primary immunodeficiency due to their role in the CD19/CD21 complex formation (17).

These mutations can lead to hypogammaglobulinaemia as a result of poor response to antigen and defective immunological memory. Researchers found changes in the constitution of B lymphocyte population and reduced amounts of switched memory B cells with high terminal differentiation potential in patients with Down syndrome (17).

CD19 has also been implicated in autoimmune diseases, including rheumatoid arthritis and multiple sclerosis, and may be a useful treatment target (17).

**CD27**

CD27 is a member of the tumor necrosis factor receptor superfamily. It is currently of interest to immunologists as a co-stimulatory immune checkpoint molecule (20).

Human CD27 are expressed on T cells, B cells, and NK cells (18).

Following antigen activation in germinal centers, B cells develop into memory B cells or plasma cells. Triggering via B-cell immunoglobulin receptors by antigens, cytokines and direct cell-to-cell contact by B and T cells plays an important role in the B cell differentiation into memory or plasma cells (20).

Adult human peripheral blood B cells are separated into three subtypes by the expression of IgD and CD27, which belong to the tumor necrosis factor receptor (TNFR) family: IgD+ CD27- naive B cells, IgD+ CD27+ and IgD- CD27+ B cells. CD27+ B cells are larger cells with abundant cytoplasm carrying somatic hypermutation, and have an ability to produce immunoglobulin, indicating that CD27 is a memory marker of B cells. The ligation of CD27 yields crucial signals that positively control the entry of B cells into the pathway to plasma cells (20).

**Nephrotic and immunity**

Numerous examples of abnormal immune responsiveness have been described in minimal change nephrotic syndrome (MCNS) the association of MCNS with allergy and with certain genetic markers of immune responsiveness, has suggested relationship between the immunological and renal abnormalities however, the nature of this relationship is uncertain (21).

The efficacy of different immunosuppressive approaches and the pivotal role of prednisone in the treatment of non-genetic forms of INS strongly implicates the immune system in the pathogenesis of this disease. However, despite extensive investigation, the mechanism by which immune dysregulation leads to disruption of the glomerular filtration barrier and consequently to proteinuria is as yet ill-defined (22).

Many studies show changes in the dynamics of T lymphocytes, especially the regulatory T cells. Alternatively, there are other reports regarding the involvement of the complement system and B lymphocytes in the pathophysiology of INS (21).

In 1974, Shalhoub postulated that MCNS is a disorder of T-cell function, resulting in increased plasma levels of lymphocyte-derived permeability factor (23).

*This hypothesis was based on the following clinical observations*
1) Remission is commonly accompanied by measles infection whereby cell-mediated immunity is suppressed.

2) MCNS is associated with Hodgkin’s disease, which is a known T-cell disorder.

3) Patients show good response to corticosteroids and cyclophosphamide, which are inhibitors of T-cell function.

4) Humoral component deposition (immunoglobulin and components) is absent in glomeruli, which is unlike that in other glomerular disorders. (24).

Therefore, the massive proteinuria and hypoalbuminemia that characterize NS were thought to result from increased glomerular capillary wall permeability due to T-cell activation triggered by several stimuli, such as viral infection or allergens (22).

Normally, cytokine release by T-cells is transient owing to the activation of Tregs that interact with T effector cells to suppress cytokine production. Tregs have been suggested to constitute a second step in an MCNS cascade, of which the first remains unclear. The induction of Treg led to a marked reduction in proteinuria in most patients with MCNS showed decreased levels of Treg (24).

Unlike role of T-cells in MCNS, which has been extensively studied, the role of B-cells is currently not well understood. Clinical trials have been conducted that demonstrated MCNS remission after B-cell depleteon using the anti-CD20 monoclonal antibody rituximab (22).

The recent successful use of anti-CD20 monoclonal antibodies for the treatment of steroid sensitive NS raises the possibility of B-cells either influencing T-cells or themselves being primary players in NS (22).

Most of these mechanisms involve immune cells and are schematized in Fig. 2

![Figure 2: Pathogenic mechanisms responsible for the disruption of the glomerular permeability barrier in idiopathic nephrotic syndrome (INS). Trigger events, such as infections, vaccination or allergens, can stimulate antigen-presenting cells (APCs) and B cells, which in turn can activate T cells by antigen presentation and cytokine production. Several T-cell alterations have been described in INS: a reduction of CD4+ Th helper (Th) cells associated with a prevalence of CD8+ cytotoxic T cells, an imbalance between Th2 and Th1 cells with an increase in the production of the Th2-specific interleukin-13 (IL-13) and a reduced frequency and function of regulatory T cells (Tregs) opposed to an increased activity of Th17 cells.

B-cell alterations have also been observed: an increased release of the soluble form of CD23 (sCD23—the immunoglobulin E receptor), a correlation between memory B-cell recovery and relapse after rituximab treatment and the presence of circulating anti-CD40 autoantibodies. In addition to the leukocyte-produced soluble factors, such as cytokines and autoantibodies, other circulating permeability proteins (i.e. hemopexin, the soluble form of the urokinase-type plasminogen activator receptor, the cardiotrophin-like cytokine factor 1, and a hyposialylated
form of the angiopoietin-like-4 glycoprotein) can directly affect podocytes, leading to foot process effacement and disruption of the glomerular permeability barrier. Furthermore, podocytes can also sense microbial products by specific toll-like receptors (TLRs) and can express costimulatory molecules, such as CD40 and B7-1 which are able to induce activation of T cells (23).

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REFERENCES


