

INTRODUCTION

In infancy and early childhood the immune system encounter antigens for the first time, mounting immune responses and acquiring memory. Young children mix with other children in families or nurseries and are exposed to many pathogens. Young children are therefore vulnerable to infection and recurrent infection is common (*Geha et al., 2007*).

When evaluating patients with recurrent infections, other factors such as parental smoking, attendance to nurseries and anatomical problems should be considered, but the critical message is to consider the diagnosis of immunodeficiency (*Vries, 2006, Geha et al., 2007*).

A survey of 10,000 American households revealed that the prevalence of diagnosed primary immunodeficiency approaches 1 in 1200. And delay in diagnosing cases with primary immune deficiency is not an uncommon finding where 32% of patients with PID were hospitalized 2 to 5 times prior to diagnosis and 10% reported hospitalization 6 to 10 times before diagnosis (*Boyle and Buckley, 2007*).

The early detection of patients with primary immunodeficiency diseases is critically important. Effective therapy is available for almost all of the different disorders, but is most beneficial when instituted before there has been damage to target organs (e.g. the lung) by infection or autoimmune

disease. Similarly, early recognition of primary immunodeficiency may lead to a precise genetic diagnosis which in turn may be important to the family in planning their future reproductive options (*Wood et al., 2007*).

Antibody deficiency syndromes make up the greatest proportion of PID diagnosis; 67% to 77% of all PIDs, as recently published by the European and Australian registries (*Eades et al., 2007, Kirkpatrick and Riminton, 2007*).

Defective antibody production causes increased susceptibility, mostly to bacterial infections that typically involve the upper and lower respiratory tract causing otitis media, sinusitis, and pneumonia but might also cause abscesses in the skin or other organs, meningitis, urinary tract infections, recurrent viral infections, and intestinal giardia species infection that can cause protracted diarrhea (*Conley et al., 2005*).

The quantitative measurement of immunoglobulin IgG, IgA, and IgM is the best test to screen for antibody deficiency states, and the pattern of their levels gives important insight into the likely etiology of the antibody deficiency (*Cunningham-Rundles, 2005*).

AIM OF THE WORK

The work aims to estimate the frequency of antibody deficiencies in pediatric patients with recurrent infections, allowing early diagnosis and rapid treatment before irreversible damage take place.

PRIMARY ANTIBODY DEFICIENCY (PAD)

Primarily immunodeficiency diseases (PID) include a heterogeneous group of disorders, which affect humoral, cellular immunity or non-specific host defense mechanisms mediated by complement proteins and cells such as phagocytes and natural killer (NK) cells (**Rosen et al., 1995**).

Antibody deficiency syndromes make up the greatest proportion of PID diagnosis; 67% to 77% of all PIDs, as recently published by the European and Australian registries (**Eades et al., 2007, Kirkpatrick et al., 2007**). Recent classification has been published by the European Society for Immunodeficiencies (ESID) online Registry as shown in table (1) (**The PedPAD Study by ESID; 2011**).

The primary antibody deficiency syndromes are a heterogeneous group of disorders in which the fundamental defect is an inability to produce an effective antibody response to a pathogen. The defect may arise from mutations in genes critical for the development and function of B-lymphocytes and sometimes they may result from probable environmental triggering in genetically predisposed individuals (**Wood et al., 2007**).

These diseases should be distinguished from a variety of other conditions inducing hypogammaglobulinemia as nephrotic syndrome, protein-losing enteropathy, malabsorption, hematologic malignancies, chemotherapy, solid-organ transplantation, infectious

diseases (e.g., human immunodeficiency virus and congenital rubella), and adverse effects of specific therapeutic drugs e.g. prolonged courses of systemic corticosteroids, anticonvulsants, antimalarial drugs, gold salts, sulfasalazine, D-penicillamine, and fenclofenac (*Ochs et al., 2004, Onigbanjo et al., 2007*).

B-cell development

Characterization of the molecular events which direct the sequential development of lymphocytes has aided in the elucidation of the pathogenesis of many primary antibody deficiencies. Studies of naturally occurring mutations and molecularly engineered mouse models have benefited this work (*Fischer and Malissen; 1998*). Haemopoietic stem cells pass through several developmental stages before mature B lymphocytes are produced. Development primarily occurs in the bone marrow compartment. Mutations in genes encoding proteins that are critical for the development of the B-lymphocyte lineage, particularly between the pro-B-cell and immature B-cell stages, may disrupt B-lymphocyte maturation leading to partial or complete developmental arrest with decreased B-lymphocyte number and profoundly reduced immunoglobulin isotype (IgA, IgM, IgG, IgD, IgE) concentrations. By contrast, mutations in key genes encoding proteins that regulate terminal differentiation and function of mature B lymphocytes may result in reduced immunoglobulin isotype concentrations usually without a change in B-lymphocyte number (Fig. 1) (*Marodi and Notarangelo, 2007*).

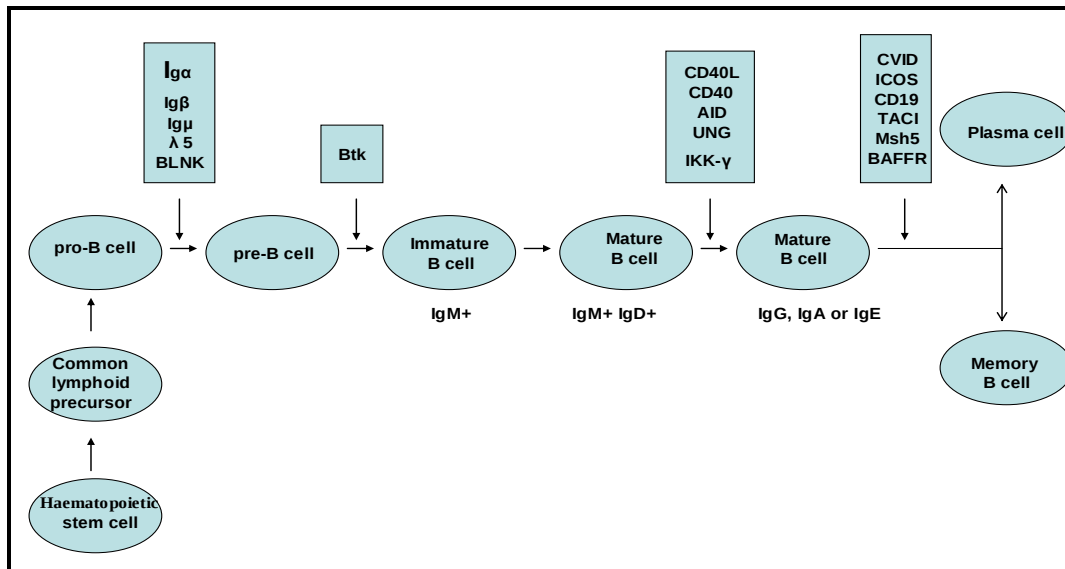


Fig. (1): B-lymphocyte development and associated primary antibody deficiencies. Igα, Igβ, μ heavy chain (Igμ), λ5B-cell linker protein (BLNK) and Bruton's tyrosine kinase (Btk) deficiencies affect early B-lymphocyte development. Deficiencies affecting terminal B-lymphocyte function include inhibitor-of-nuclear-factor-κ B kinase (IKK-γ), conditions causing hyper-IgM syndrome (CD40 and CD40 ligand (CD40L)), activation-induced cytidine deaminase (AID and uracil-DNA glycosylase (UNG) deficiencies), and disorders associated with common variable immunodeficiency (CVID) including inducible costimulator (ICOS), CD19, transmembrane activator and calcium-modulating cyclophilin-ligand interactor (TACI), homolog of Escherichia coli MutS (Msh5), and possibly B-cell-activating-factor receptor (BAFFR).

(Marodi and Notarangelo, 2007).

Table (1): Predominantly antibody disorders.

Sub-category	Sub-registry	Mutation Entries
Agammaglobulinemias	Agammaglobulinemia	Btk. (x-linked), BLNK/SLP65, CD79A, IGLL1, IGHM, LRRC8, none/unknown
Class switch recombination defects (CSR) / HIGM syndromes	Activation-induced cytidine deaminase deficiency (AID)	AID
	CD40 deficiency (TNFRSF5)	CD40 (TNFRSF5)
	CD40 ligand deficiency (CD154)	CD40L (CD154)
	CSR defects and HIGM syndromes with unknown genetic cause	none/unknown
	PMS2 deficiency (HIGM phenotype)	PMS2
	Uracil-DNA glycosylase deficiency (UNG)	UNG
Hypogammaglobulinemias	Common variable immunodeficiency (CVID)	BAFFR, CD19, CD21, CD81, ICOS, TACI, none/unknown
	Deficiency of specific IgG	none/unknown
	Dystrophia myotonica type2 (PROMM/ZNF9)	ZNF9
	IgA with IgG subclass deficiency	TACI, none/unknown
	Immunoglobulin chain deficiencies	Heavy chain, Kappa light chain, Lambda light chain
	Isolated IgG subclass deficiency	BAFFR, CD19, CD21, CD81, ICOS, TACI, none/unknown
	Other Hypogammaglobulinemias	none/unknown
	Other immunoglobulin gene deletions	none/unknown
	Secondary hypogammaglobulinemia	none/unknown
	Secondary selective IgA deficiency	none/unknown
	Selective IgA deficiency	TACI, none/unknown
	Selective IgM deficiency	none/unknown
	Thymoma with immunodeficiency	none/unknown
	Transcobalamin II deficiency	Transcobalamine II
	Transient hypogammaglobulinemia of infancy	none/unknown

(The PedPAD Study by ESID; 2011)

Agammaglobulinemia

Agammaglobulinemias are rare antibody deficiencies caused by defects in early B-cell development and characterized by low numbers of or absent B cells, marked hypogammaglobulinemia, and increased susceptibility to infections. Agammaglobulinemias include two main categories, x-linked (XLA) and autosomal recessive (ARA) (**Fried and Bonilla, 2009**).

XLA accounts for 85% of known cases of agammaglobulinemia and is caused by a deficiency of the Bruton tyrosine kinase (BTK) (**Conley et al., 2005**).

The incidence of XLA in the United States has been estimated to be at least 1/190,000 male births. The age of onset of symptoms for most patients is between 3 months and 3 years, with over 50% of patients becoming symptomatic by 1 year of age and more than 90% of patients becoming symptomatic by 5 years of age (**Winkelstein et al., 2006**).

T-cell numbers and function are normal for patients with XLA. Neutropenia, often severe, is found in 15 to 25% of patients at the time of diagnosis. Neutropenia appears to be a result of the patient's high bacterial burden related to associated infections especially that it has only been observed in the presence of infections and it appeared to resolve with antimicrobial and immunoglobulin therapy (**Farrar et al., 1996**).

Patients with XLA develop together with repeated infections non-infectious complications as juvenile rheumatoid arthritis, aseptic polyarthrititis, sensorineural hearing loss, and colorectal cancer (**Berlucchi et al., 2008, Brosens et al., 2008**). Autoimmune diseases such as Crohn's disease and type I diabetes mellitus have been described in a few patients with XLA (**Bonilla and Geha, 2004**). The occurrence of a dermatomyositis-like syndrome, marked by peripheral edema, erythematous rash, and evidence of inflammation of the skin and muscle on biopsy specimens, has been associated with disseminated enteroviral disease in patients with XLA (**Mckinney et al., 1987**)

At the time of diagnosis, most, but not all, patients have low serum immunoglobulin levels, with IgG levels of <200 mg/dl, IgA levels of <15 mg/dl, and IgM levels of <40 mg/dl, Almost all patients with classic XLA have markedly decreased B-cell numbers, with <2% of CD19- or CD20-positive lymphocytes in the blood. B-cell lymphopenia with normal T-cell numbers can be considered diagnostic in the context of a positive family history or if the mother is an identified carrier for the disease (**Winkelstein et al., 2006**).

In 10% of cases, agammaglobulinemia is not due to mutations in *BTK*; rather, it is inherited as an autosomal trait and is therefore classified as autosomal-recessive agammaglobulinemia (ARA). Special genetic defect has not been yet identified in 5% of these cases. They have the same

phenotype as XLA. ARA is suspected in females with agammaglobulinemia, in families with an autosomal pattern of inheritance, if consanguinity is present, or if a *BTK* mutation has been excluded for an affected male (*Bonilla et al., 2005*).

Immunodeficiency with Low IgG and Normal or High IgM Levels (Class Switch Recombination Defects)

The term hyper-IgM has been used to describe a rare group of disorders of class switch recombination defect (CSR) with characteristically elevated IgM levels and low levels of switched isotypes (IgG, IgA, and IgE). The molecular defects for the majority of these disorders have been identified, and their characterization has been instrumental in elucidating some of the central mechanisms of antibody isotype switching and somatic hypermutation (SHM) (*Fried and Bonilla, 2009*).

X-linked hyper-IgM is caused by mutations in the gene encoding CD40L. A rare autosomal recessive form with an identical phenotype is due to mutations in the gene encoding CD40. Defective CD40L or CD40 expression results in a combined humoral and cellular immunodeficiency with absent or abortive germinal center formation as well as impaired interactions of T cells with dendritic cells (*Notarangelo et al., 2006*).

Patients usually present in infancy with severe and/or recurrent bacterial respiratory and gastrointestinal infections as well as opportunistic infections such as *Pneumocystis jirovecii*

pneumonia, disseminated fungal infections with *Candida*, *Cryptococcus* and *Histoplasma*, disseminated Cytomegalovirus (CMV) and Herpes simplex virus infections, and cholangitis due to *Cryptosporidium parvum*. Other clinical features are neutropenia, chronic anemia associated with Erythrovirus (Parvovirus B19) infections, and an increased incidence of gastrointestinal tumors (*Winkelstein et al., 2003*).

Another forms of hyper-IgM with autosomal-recessive inheritance are caused by deficiencies of activation-induced cytidine deaminase (AID) or uracil-DNA glycosylase (UNG), resulting in profoundly defective CSR. These patients present with recurrent or severe infections. Two-thirds of these patients have lymphoid hyperplasia with prominent cervical lymph nodes and tonsillar hypertrophy. Pathology usually reveal massive germinal center enlargement. Patients also have an increased incidence of autoimmune disease such as autoimmune hemolytic anemia and autoimmune thrombocytopenia (*Quartier et al., 2004*).

Another phenotype of CSR defect occurs due to mutations in the genes encoding the I- κ B kinase γ chain (also called NF- κ B essential modulator {NEMO}). NEMO patients have variable manifestations of ectodermal dysplasia as conical or absent teeth, sparse hair, frontal bossing, and decreased eccrine sweat glands together with increased susceptibility to mycobacterial infections. However, these defects include significant elements of T-cell and NK cell dysfunction and are

best classified among the combined (cellular and humoral) immunodeficiencies (*Jain et al., 2001, Orange et al., 2003*).

Diagnosis of hyper IgM requires presence of low IgG and IgA levels, and normal to elevated IgM levels (*Winkelstein et al., 2003*). Among those patients with AID and UNG deficiency IgG and IgA levels are generally profoundly decreased (IgG levels of <200 mg/dl and IgA levels of <20 mg/dl), and IgM levels are normal to increased (100 to 3,700 mg/dl) (*Quartier et al., 2004*). Specific immunoglobulin responses are poor for all subtypes of CSR defects. Lymphocyte subset composition is normal. T-cell proliferative responses are normal except for patients with CD40L and CD40 deficiencies, who have impaired responses to recall antigens. CD40 and CD40L expression can be evaluated by flow cytometric analysis. Gene testing for mutations in CD40, CD40L, AID, UNG, and NEMO is available (*Bonilla et al., 2005*).

Common Variable Immunodeficiency (CVID)

CVID is a heterogeneous disorder of B-cell differentiation and maturation with dysfunctional antibody production, with an estimated prevalence of 1 in 25,000 to 1 in 75,000 individuals (*Hammarstrom et al., 2000*).

While the etiology remains unknown for the majority of patients, gene defects have been detected in approximately 10 to 15% of CVID patients in recent years. Reported defects involve B cell activation (CD19 and CD81 deficiency) (*Van*

Zelm et al., 2010), inducible costimulator (ICOS) deficiency (*Grimbacher et al., 2003*) and B cell activating-factor receptor (BAFF-R) deficiency (*Warnatz et al., 2009*). Moreover, genetic defects have been identified that do not cause hypogammaglobulinemia, but increase disease susceptibility as transmembrane-activator and calcium modulating cyclophilin-ligand interactor (TACI) deficiency (*Driessen & Van der Burg, 2011*).

The age of presentation of CVID varies widely. According to a recent large European registry study of 334 patients, the most common age at the onset of symptoms was in the third decade, with a mean of 26.3 years and a median of 24 years. This indicates that many patients suffer from their disease for many years before their immunodeficiency is recognized (*Chapel et al., 2008*).

Patients often come to medical attention due to acute or chronic bacterial and, less frequently, viral infections. Noninfectious complications are also frequent, encompassing lymphoproliferative disease, granulomatous disease, a spectrum of gastrointestinal disorders (*Cunningham-Rundles and Bodian, 1999*).

Hypertrophy of lymphoid tissue is common in CVID. Splenomegaly has been reported in 26% of patients (*Quinti et al., 2007*).

Granulomatous disease is present in 11.6 % of patients with CVID. Granulomas are of the noncaseating type and resemble those found in sarcoidosis. In many cases, patients are misdiagnosed with sarcoidosis before their antibody deficiency is detected and the diagnosis of CVID is made. Although granulomas most commonly affect the lungs, they are also found in almost any other organ including skin, liver, spleen, bone marrow, and the gastrointestinal tract of patients (**Wehr et al., 2008**).

Studies have found no increased risk of malignancies among relatives of patients with CVID, suggesting that the immunodeficiency itself predisposes one to cancer (**Mellemkjaer et al., 2002**).

Autoimmune diseases as idiopathic thrombocytopenic purpura, autoimmune hemolytic anemia, rheumatoid arthritis, and pernicious anemia are present in 20 -30 % of these patients (**Quinti et al., 2007**).

CVID remains predominantly a diagnosis of exclusion. Other specific immunodeficiency diagnoses have to be ruled out by careful evaluation of clinical features and laboratory phenotypes. For patients with CVID, IgG levels are reduced by greater than 2 standard deviations (SDs) below the mean for age. An associated reduction in either the IgA or IgM level has been included as part of the diagnostic criteria by some authors (**Fried and Bonilla, 2009**).

In CVID the evidence of impaired specific antibody responses to infection or vaccine challenge is essential for diagnosis. Peripheral B-cell numbers can be either normal or reduced. T-cell numbers and function are also reduced in some patients. In some cases, molecular testing may be required to distinguish other diseases with similar phenotypes, including CSR defects, agammaglobulinemia, and X-linked lymphoproliferative syndrome (*Eastwood et al., 2004*).

The number of isotype-switched memory B cells in the peripheral blood has been found to be one of several useful parameters to distinguish distinct phenotypes of CVID. Reduced numbers of switched memory B cells (<2% of total B cells) have been shown to correlate with disease-associated complications such as splenomegaly and granulomatous disease. One study reported a significant correlation with autoimmune disease if the cutoff level for switched memory B cells was lowered to <0.55% of total B cells (*Sanchez-Ramon et al., 2008, Wehr et al., 2008*).

Selective IgA Deficiency

Selective IgA deficiency is a common immunological variant with a prevalence of 1:400 to 1:600 in the healthy U.S. population. There is familial clustering of IgA deficiency with CVID, with 20 to 25% of individuals having a positive family history of either IgA deficiency or CVID. Some IgA deficient patients progress over time to CVID (*Fried and Bonilla; 2009*).