Common Variable Immunodeficiency: Epidemiology, Pathogenesis, Clinical manifestations, Diagnosis, Classification and Management

Running title: Common Variable Immunodeficiency

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Abstract
Common variable immunodeficiency (CVID) is a heterogeneous disorder characterized by hypogammaglobulinemia and increased susceptibility to recurrent bacterial infections. It is the most frequent symptomatic antibody deficiency with a wide variety of infectious and non-infectious complications. Numerous studies demonstrated that different immunological and genetic defects are involved in the pathogenesis of CVID. However, in most cases, the genetic background of the disease remains unidentified. This review aims to discuss different aspects of CVID including epidemiology, pathogenesis, symptoms, diagnosis, classifications and management of the disease.

Key words: Common variable immunodeficiency; epidemiology; pathogenesis; symptoms; diagnosis; classifications; management.

Resumen
La inmunodeficiencia variable común (CVID) es un trastorno heterogéneo caracterizado por una hipogammaglobulinemia y por una mayor susceptibilidad a infecciones bacterianas recurrentes. Se trata de la inmunodeficiencia humoral sintomática más frecuente y cursa con una extensa variedad de complicaciones infecciosas y no infecciosas. En la patogénesis de la CVID están involucrados diferentes defectos inmunológicos y genéticos. Sin embargo, en la mayoría de los casos, el fondo genético de la enfermedad permanece sin identificar. Esta revisión tiene como objeto discutir diferentes aspectos de la CVID, incluyendo epidemiología, patogénesis, síntomas, diagnóstico, clasificaciones y tratamiento de la enfermedad.

Palabras clave: inmunodeficiencia variable común; epidemiología; patogénesis; síntomas; diagnóstico; clasificaciones; tratamiento.
1. Introduction

Common variable immunodeficiency (CVID) is the most common symptomatic primary immunodeficiency (PID) characterized by hypogammaglobulinemia and impaired specific immunoglobulin (Ig) production. Patients with CVID present a broad range of clinical manifestations including recurrent bacterial infections, autoimmunity, interstitial lung disease, enteropathy, lymphoproliferation, malignancy and allergic diseases [1, 2]. In recent years, several monogenic defects involved in the presentation of CVID have been identified; however, these genetic defects account for less than 20% of CVID patients in non-consanguineous cohorts [3] and approximately 70% of CVID patients in consanguineous cohorts [4]. Furthermore, several abnormalities in the innate and adaptive immunities have been reported [5-7], however, the exact molecular defects leading to CVID is still unknown.

Based on various clinical manifestations and immunological data, some classifications have been defined for CVID patients [8]. Since CVID is considered as a heterogeneous group of PIDs with various clinical and immunological features, an appropriate classification for these patients is essential. Regarding diagnosis of CVID, knowing clinical features along with immunological and genetic analysis are most important. Clinical heterogeneity in CVID patients has led to diagnostic challenges and difficulties in determining the optimal treatments for patients [9]. Although immunoglobulin therapy is the mainstay of the treatment for CVID patients, hematopoietic stem cell transplantation (HSCT) is utilized for CVID patients mostly associated with cellular immune defects and therapy resistant autoimmunity. HSCT a potentially curative treatment approach has been performed in some cases of CVID with mixed results. Few studies also showed the advantage of new immunomodulation via targeted treatment in a selected group of CVID patients with specific genetic defects [10].

The aim of this review is to present a comprehensive view of common variable immunodeficiency including Epidemiology, Pathogenesis, Clinical manifestations, Diagnosis and also Classification of the disease.

2. Epidemiology

Antibody deficiencies are the most common defect among primary immunodeficiencies and account for 30-70% of all patients identified with a specific defect. Within the PID group, CVID is the most frequent symptomatic antibody deficiency [11]. According to a recent report by the Jeffrey Modell
centers network, there are important geographic disparities in terms of CVID prevalence for North America (n=6443), Europe (n=4279), Asia (n=459), Australia (n=657) and Africa (n=156) [12]. The highest prevalence of CVID has been documented in the USA (40.2% of all PID patients) whilst the lowest rates were observed in Middle East countries 2.6% and Africa with only 1.3% [12]. The most likely reasons responsible for these differences are the availability of appropriate diagnostic methods, registry and PID awareness [13].

In CVID the age of onset is associated with the predominant clinical manifestations. The United States Immunodeficiency Network (USIDNET) database compared patients diagnosed in the pediatric age (17 years and younger) to patients with adult onset (18 years or older) CVID. Based on this epidemiological study (n=457 patients), otitis media, developmental delay and failure to thrive were more frequent in pediatric-onset CVID patients, whilst bronchitis, arthritis, and fatigue were more common in adult CVID patients [14].

3. Pathogenesis
In previous years, various studies have investigated the pathogenesis of CVID. The identification of monogenetic CVID causes has increased our understanding of this complex disease [9, 15]. In addition, recent studies demonstrated a role of epigenetic modifications in the development of CVID disorder [16, 17]. Gene mutations at three levels of the surface, cytoplasmic, and nuclear of cells have provided in Tables 1, 2 and 3, respectively.

3.1. Gene and Molecule defects
Different members of the Tumor necrosis factor (TNF) receptor superfamily have been reported to be involved in the pathogenesis of CVID. In this pathway, single gene defects in trans membrane activator and calcium modulator and cyclophilin ligand interactor (TACI, encoded by TNFRSF13B), B cell activating factor belonging to the tumor necrosis factor family BAFF receptor (BAFF-R, encoded by TNFRSF13C), TNF-like weak inducer of apoptosis (TWEAK, encoded by TNFRSF12) and CD27 encoded by TNFRSF7 have been described. BAFF-R and TACI, are known to participate in B cell development and activation by engaging a proliferation-inducing ligand (APRIL) and/or BAFF [18, 19]. Although studies have demonstrated that 8% to 10% of CVID patients have a defect in TACI, these mutations have been also described in the general population without hypogammaglobulinemia raising the question of their pathologic impact. Since TACI regulates the function of the B-cell receptor (BCR), Toll-like receptor (TLR) 7 and 9 molecules, defects in molecules could result in impairment of B cell activation/maturation, which then may contribute to the development of
autoimmune manifestation [18, 20]. In addition to the TNF receptor superfamily, defects in the CD19 complex (CD19, CD21, and CD81) or co-stimulatory molecules such as CD20 and IL-21 receptor have also been described in CVID patients. These molecules are important for the adequate development, maturation and survival of B cells and are likely involved in the CVID pathogenesis [5]. Furthermore, defects in two co-stimulatory and inhibitory receptors located on T cells (inducible costimulator, ICOS and cytotoxic T-Lymphocyte Associated Protein 4, CTLA-4) were identified in a group of CVID-like patients with associated T cell abnormalities [5].

The last years have revealed defects in several signaling-associated molecules at three levels of the surface, cytoplasmic, and nuclear molecules implicated in the pathogenesis of CVID patients. The detailed functions and effects of the most important defects are summarized in Tables 1, 2 and 3. Nowadays, each defective molecule is considered as a separate form of immunodeficiency and is categorized as monogenic disorders. In this sense, IUIS (International Union of Immunological Societies) classification classified PID disorders in the specific categories [21].

### 3.2. Epigenetic changes

In recent years several studies have highlighted the role of epigenetic factors in the pathogenesis of CVID [16, 17, 22, 23]. Epigenetic mechanisms can influence gene expression without altering the germ-line DNA gene sequences and play an important role in the normal developmental program of immune cells [16]. The mechanisms described so far, include DNA methylation, chromatin modulation, histone modification, transcription factor expression and non-coding RNAs (ncRNAs) [24].

DNA methylation is catalyzed by DNA methyltransferases (DNMTs) and represses gene expression by reducing transcription factors and DNA regulatory elements or by making DNA fragments inaccessible to transcription factors [25]. This mechanism plays an important role in both, early and late stages, of B cells development. The study of twins discordant for the CVID diagnosis revealed a higher degree of DNA methylation in the switched and non-switched memory B cells of the patient when compared to the healthy sibling. Furthermore, hypermethylation of genes such as **PIK3CD**, **BCL2L1**, **RPS6KB2**, **TCF3**, and **KCNN4** in B cells was observed and demethylation during the transition from naive to memory cells was impaired. This observation revealed a novel mechanism responsible for the defective generation of memory cells in CVID patients [17]. Using an equine CVID model, methylation disturbance in the form of hypermethylation of **PAX5** was shown to block B cell development, to reduce B cell numbers and finally to the development of late-onset CVID [22].
Other factors implicated in the epigenetic regulation of B cells are non-coding RNAs (ncRNAs) [26]. ncRNAs exert their regulatory functions by mRNA post-transcriptional changes or influencing DNA transcription [26]. miRNAs, a subgroup of short ncRNAs that mainly represses gene expression, contribute to the regulation of different stages of B cells development. Studies on the role of miRNAs in the pathogenesis of human CVID are still ongoing; however, mouse models have already demonstrated the importance of these molecules for T and B cell development [27]. Importantly, knockout mice lacking miR-142 [23] or miRNA-155 [27] exhibit immunological features similar to those observed in CVID patients.

Histone and chromatin modifications are an epigenetic mechanism that might be also involved in the CVID pathogenesis. Defects in histone and chromatin modification enzymes have been described in patients with Kabuki syndrome, a complex multi-system syndrome that includes hypogammaglobulinemia, reduced naïve and switched memory B cells and an increase of a CD21_{low} B cell population features often found in patients with CVID. In the Kabuki setting, the B cell differentiation defect was shown to be associated with an impaired histone modification process which is fundamental for the correct B cell development [28].

### 3.3. Microbiome dysbiosis.

The human microbiome interacts with the systemic immune system via immune cells and some bacteria that may cross the gut epithelium, exposing the systemic immune system to microbial components [29]. Bacterial products such as lipopolysaccharide (LPS) can activate the immune response through recognition of microbe-associated molecular patterns by the innate immune system [30]. Microbial dysbiosis may lead to the overgrowth of proinflammatory bacteria or a decrease of anti-inflammatory bacteria which subsequently leads to a further imbalance of the immune system [31].

Importantly CVID patients have extensive microbial dysbiosis with a reduced alpha diversity and differences in the taxonomic profile when compared to patients with inflammatory bowel disease (IBD). This reduction of alpha diversity in CVID patients is associated with raised T-cell activation markers including LPS and sCD25 and decreased levels of plasma IgA, suggesting that the altered gut microbiota profile could modulate the gut permeability with subsequent LPS and sCD25 elevation and decreased IgA level along with chronic immune activation in CVID [32]. Nevertheless, attributing causality to microbial changes due to the presence of low IgA level and impaired epithelial gut barrier remains challenging and further research in this field is clearly needed.
4. Immune cell abnormalities
Defects in the adaptive and innate immune system and in particular abnormal B cell and T cell numbers have been reported in CVID patients. Although the classic immunological defect in CVID is related to plasma cell abnormalities, alterations of other B cells subsets are not uncommon. Table 4 gives an overview of the so far described immune cell abnormalities in CVID.

4.1. B cell subsets
B cell development involves sequential steps of maturation initiated in the bone marrow and completed in peripheral compartments. Immature B cells pass through transitional stages and become either marginal zone B cells or follicular naïve B cells in the periphery. In germinal centers, follicular B cells differentiate to switched memory B cells and antibody secreting plasma cells, whereas marginal zone B cells evolve to IgM memory B cell [33, 34]. The majority of studies demonstrated that almost 90% of CVID patients have normal B cell counts, [35, 36], indicating that the major defect is likely related to the alterations of the terminal stages of B-cells differentiation. Furthermore, several groups including our own have shown that disturbed B-cell subsets could be the result of an increase of terminal B-cell apoptosis [37, 38]. The impaired antibody production despite normal B cell counts also suggests a defect in the differentiation of B cells into memory and plasma cells in many CVID patients [35].

Several studies have reported decreased IgM memory B cells (CD19+/CD27+), class switched memory B cells (CD19+/CD27+/IgD-/IgM-) and plasma cells in CVID patients [34, 39, 40]. Since the correct germinal center formation is essential for the development of switched memory B cells in secondary lymphoid organs, it seems that the reduction of switched memory B cells is highly related to the impaired germinal center reaction. In contrast, a profound expansion of the transitional B cell pool has been observed in two siblings with CVID disorder (especially BAFF-R deficiency) due to a block in the transition from T1 to T2 cells [41-43]. Furthermore, it has been reported that a subgroup of CVID patients manifests with the expansion of a special subset of B cells called CD21low B cells. This subset is distinct from other B cell subsets as it shows low expressions of CD21 and CD38 simultaneously [43]. In one study, it has been demonstrated that its expansion is associated with an abundance of IFN-γ producing CD4+ CXCR5+ follicular T helpers (TFH) cells and immune dysregulation in CVID patients [44].
The characterization of signaling pathways essential for B-cell differentiation and class-switch recombination (CSR) have been evaluated by Taraldsrud et al. [45]. In this study constitutive phosphorylation levels of signal transducer and activator of transcription (STAT 3, -5 and -6), phosphoinositide phospholipase C-γ, Erk, and Syk were significantly increased in B cells of selected CVID patients with non-infectious complications. In the future, the combination of surface marker determination together with kinase phosphorylation pattern may allow developing a model able to predict the occurrence of non-infectious complications in CVID patients.

4.2. T cell subsets

T cell abnormalities have been reported in CVID patients. These abnormalities are broad and include total numbers, percentages, surface markers and function of different T cell subpopulations [5]. Some studies showed a reduction of the total, naïve and memory CD4+ T cells, recent thymic emigrants (RTE) and an increase in activated CD4+ T cells [46]. Decreased thymic output, enhanced T cell turnover and spontaneous apoptosis lead to CD4+ T cell count reduction [46], whereas the observed increase of activation of CD4+ T cells might be due to low regulatory B cell (Breg) numbers and defective Breg responses after T-cell stimulation [47]. It has been demonstrated that CVID patients with a profound reduction in CD4+ T cell counts are more likely to develop autoimmunity and lymphoproliferation, indicating that there is a strong correlation between the frequency of naïve CD4+ T cells and clinical manifestations. Despite T cell abnormalities reported in CVID patients, however, based on discrepancies of different criteria for diagnosis, it is not clear whether patients with such defects should be considered affected with CVID or with late onset combined immunodeficiency (LOCID) or other forms of combined immunodeficiencies [48, 49].

There is still a controversy regarding the role of the different T helper subsets for the pathophysiology or development of clinical manifestations in CVID patients, it has been reported that type 2 helper (TH2) cytokine levels such as IL-4 and IL-10 are significantly elevated in CVID patients, whilst this increase was not observed for TH1 cytokines [50]. Similarly, it has been reported that the serum level of CD30 (an indicator of TH2 cytokine production) is increased in CVID patients [50], suggesting that CVID patients might be skewed towards TH2 responses. However, other studies report excessive TH1 responses in CVID patients [51, 52]. This discrepancy is likely due to heterogeneous CVID etiologies, differences in the composition of patient cohorts and experimental methodology.
TH17 cells have also been investigated in CVID patients. TH17 cells and its related cytokines IL-17A, IL-17F, IL-22 and IL-21 are involved in the host defense against extracellular bacterial and fungal infections and also play an important role in inflammatory diseases. Barbosa et al. reported a decrease in the frequency of circulating TH17 cells in CVID patients [53]. Moreover, another study demonstrated a reduction of TH17 cell–specific gene expression in CVID patients when compared to the healthy controls [54]. A negative correlation between TH17 cells, probably due to its regulatory role on the appropriate function of the germinal center, and the expansion of activated CD21\textsuperscript{low} B cells has been observed [36, 43, 44]. Regarding TFH in CVID patients, some studies have identified increased TFH counts [55, 56], whereas a decrease in TFH cells has been observed in both ICOS-deficient mice and patients [57, 58]. Since TFH cells express ICOS in its surface, decreased TFH counts are the logical consequence in patients with ICOS deficiency.

Regulatory T cells (Tregs) are important regulators of the immune system and play a crucial role in the maintenance of self-tolerance [59]. Although Kutukculer et al. did not observe significant differences between the percentages and absolute counts of Treg in CVID patients when compared to healthy controls and therefore concluded that Treg cells are not relevant for the pathogenesis of CVID [60] whereas the results of other studies suggest that a reduction in the Treg counts can influence disease manifestations and identified a correlation between Treg counts and autoimmune manifestation, granulomatous lesion and splenomegaly [61, 62]. Furthermore, it has been showed that sorted Treg cells from patients with CVID are less effective in suppressing proliferation of autologous and allogenic effector CD4\textsuperscript{+} T cells than CVID patients without autoimmunity [63] and that the decrease of Treg cells correlates with the expansion of CD21\textsuperscript{low} B cells in CVID patients with autoimmunity [64].

Similar to CD4\textsuperscript{+} T cells, a decline in the frequency of CD8\textsuperscript{+} T cells subsets has been demonstrated. Naïve and effector memory CD8\textsuperscript{+} T cells numbers are reduced whereas higher percentages of activated CD8\textsuperscript{+} T cells have been reported [48, 65, 66]. Studies showed that CD8\textsuperscript{+}HLA-DR\textsuperscript{+}, CD8\textsuperscript{+}CD38\textsuperscript{+}, and CD8\textsuperscript{+}CD38\textsuperscript{+}HLA-DR\textsuperscript{+} T-cell counts are higher in CVID patients and also that this increase is restricted to patients with clinical complications including autoimmune disease, splenomegaly, lymphoid proliferation, and granulomatous disease [65]. Moreover, higher expression levels of Granzyme B in CD8\textsuperscript{+} T cells correlate with autoimmune manifestations in CVID patients [67]. Viallard et al showed that patients with low CD27\textsuperscript{+} B cells had higher percentages of HLA-DR CD8 T\textsuperscript{+} cells with a differentiated effector phenotype [68]. These data further confirm a higher activation status of CD8\textsuperscript{+} cells in CVID patient [69]. Finally, Paquin-Proulx et al. found a specific subset of γδ T
cells expanded in CVID patients. Although they suggested that this deviation in γδ T cell subsets is a general feature of CVID patients further studies are needed to confirm this observation [70].

4.3. Dendritic Cells (DC)

Dendritic cells play an important role in the induction of T cells responses as well as in the differentiation of naïve B cells to plasma cells. Studies about dendritic cells in CVID patients have shown a progressive decline of these cells as well as maturation and function abnormalities [71]. In this regard, the expression of maturation and costimulatory molecules such as CD80, CD86 and HLA-DR and also the production of IL-12 were lower in CVID patients [71, 72]. Furthermore, decreased IFN-α production upon TLR-9 stimulation in plasmacytoid dendritic cells has been demonstrated [63, 73]. In contrast, Taraldsrud et al. reported that DCs of CVID patients have a normal response to TLR-7 and TLR-9 and viral stimulation as well as have normal numbers of DC progenitor cells in the bone marrow [74]. Overall, based on the prominent role in presenting antigens to T cells and initiation of primary immune responses, abnormalities in DCs may lead to a defect in the generation of antigen specific CD4+ T cells and also impaired antibody production as those seen in CVID patients.

4.4. Monocytes/Macrophages

Monocytes from CVID patients exhibit significantly increased reactive oxygen species (ROS) generation which might result in specific clinical manifestation including malignancies, autoimmune disorders, and some acute and chronic pulmonary diseases [75]. Aukrust et al. suggested that persistently increased TNF levels and TNF receptor expression might contribute to the activation of monocytes/macrophages [76]. Moreover, it has been demonstrated that increased IL-12 production in CD14+ monocytes results in skewing T cell responses toward TH1. In addition, overexpression of IL12 leads to the upregulation of IFN-γ in T cell subsets and subsequently skews the immune system away from the TH2 responses to TH1 [77]. Thus, it seems that the altered cytokine profile in monocytes is a probable mechanism contributing to the enhanced TH1 profile and by this to defective antibody production in a selected group of CVID patients.

In the absence of additional cytokines, the tendency of monocytes (mainly CD83 negative) from CVID patients to form giant cells is almost twice as high when compared to normal cells. However, the excess of cytokines such as IL4, GMCSF, IFNγ, TNFa contribute to 5 fold increased monocyte fusion index in CVID. A higher fusion index contributes to granuloma formation (a lymphoproliferative complication) and is related to chronic inflammation (e.g. inflammatory cytokines, particularly IFNγ and TNF) and lymphocyte concentration. Of note, in vitro the fusion rate of monocytes treated with Ig
products can be increased which raises the debate of whether this standard treatment is involved in the risk of granulomatous disease, possibly by enhancing FcγRI expression [78].

4.5. Innate lymphoid cells (ILC)

Innate lymphoid cells (ILC) are a group of immune effector cells characterized by lymphoid morphology but lacking the B and T cell receptor. They play an important role in innate immunity, tissue development and cytokine production [79]. Based on phenotypic and functional characteristics, ILCs are classified into three major groups. Group 1 comprise NK cells and other noncytotoxic ILCs, defined by T-bet expression and IFN-γ production; group 2 express GATA-3 and produce type 2 cytokines such as IL-4, IL-5 and IL-13; group 3 contain the transcription factor RORγT and are capable to produce IL-17 and IL-22 [79]. A recent study demonstrated that the numbers of IL-17+CD127+Thy-1+ Lin- ILCs are decreased in CVID patients [54]. Conversely, another study indicated a significantly expanded population of Lin-CD127+ cells producing IFN-γ, IL-17A and IL-22 and it has been suggested that the expansion of these cells is characteristic for CVID patient with inflammatory manifestations [80].

Regarding the ILC1 group, the frequency and function of NK cells have been also evaluated in CVID patients. NK cells are a component of the innate immune system and play an important role in the killing of the tumor and virally infected cells [81, 82]. Kutukculer et al. reported increased numbers of CD3−CD16+CD56+CD28+ NK cells whilst CD28- NK cells were significantly decreased in CVID patients. This augmentation could be related to the presence of a compensatory mechanism for protection against tumor development and viral infections [83], as high frequency of bacterial infections and non-infectious disease especially viral infections and tumor development have been observed in CVID patients with NK deficiency [82].

CD117+ILCs group 2 have a role in the generation of antibody production. It has been recently reported that the number of these cells are reduced in patients with CVID (with impaired response to IL-2, -7, -25 and -33) [84]. These patients manifest with an increased prevalence of chronic enteropathy and an immunologic profile of lower numbers of peripheral marginal zone-like B cells [84].

4.6 NKT

NKT cells are a group of lymphocytes with a rearranged Va14-Ja18 TCR that recognizes glycolipids and are able to produce TH1 and TH2 cytokines upon stimulation. In a study by Carvalho et al, it has
been demonstrated that there is a dysbalance of NKT cell subsets in CVID patients as the frequency of CD4⁺ NKT cells is higher than CD8⁺ NKT cells [85]. However, they found that there was no difference regarding the frequency of circulating NKT cells between the patients and healthy control. In contrast, a study on iNKT cells identified a marked decrease in the proportion of these cells and was associated with low or absent switched memory B cells, supporting a potential correlation between iNKT cell and B cell function [86].

5. Clinical manifestations

5.1. Infections

Infections are the most typical clinical manifestations in CVID patients and involve the respiratory and gastrointestinal system [87]. The upper and lower respiratory tract are the most common side of infections and contribute significantly to morbidity and mortality of CVID patients [88]. Bacterial infections due to Streptococcus spp., Haemophilus spp., Moraxella catharralis, Neisseria meningitides, and Staphylococcus spp. are the major group of pathogens affecting CVID patients [88, 89]. Moreover, viral pathogens such as Rhinovirus and Herpes zoster and Mycoplasma spp. are more prevalent and even more persistent in CVID [90]. Although opportunistic infections including Pneumocystis jirovecii and Cytomegalovirus are not characteristic for CVID and should challenge this diagnosis, they can be found in a subgroup of patients with CVID diagnosis and low CD4⁺ T-cells [91].

Bronchiectasis and interstitial lung disease are two important lung complications that manifest after recurrent and severe lung infections [92, 93]. Also, recurrent sinusitis, bronchitis and otitis media are found in half of the patients. Ig replacement has an important role in decreasing the infections but yet susceptibility to infections remains problematic [89]. Ig replacement treatment modifies the natural course of the disease, but mainly in terms of invasive infections, while the incidence of respiratory infections, including pneumonia may depend on several factors, such as late diagnosis with the development of bronchiectasis and immune dysregulation [88].

Gastrointestinal tract infections manifest in form of chronic or acute diarrhea [94]. Typical histopathologic findings are in the intestinal tissue of CVID patients are deep follicular lymphoid hyperplasia and reduced plasma cell numbers. Giardia lamblia followed by Campylobacter jejuni and Salmonella spp are the most common identified pathogens. These infections are characteristic for
patients with undetectable serum IgA levels and intravenous Ig replacement therapy has failed to show consistent improvement of gastrointestinal symptoms [95, 96].

Despite the known increased infection susceptibility, there is no significant difference between the prevalence of *Helicobacter pylori* infection in CVID when compared to healthy individuals. One potential explanation is the relatively common prescription of prophylactic and therapeutic antibiotics in this patient group that may collaterally prevent *H pylori* infections [95, 97, 98]. However, *H pylori* infection in CVID patients should be considered and screening for CVID patients due to its role in development gastric dysplasia or gastric cancer [96, 99].

Regarding mild infections in CVID patients, a group of patients only experience infections in their life and no non-infectious complications. According to clinical phenotype classification by Chapel et al, these patients are categorized as infection only phenotype. A milder clinical severity and longer survival in these CVID patients could be due to the presence of a compensatory mechanism in memory B cell generation and IgG production after co-culture of PBMC with anti-CD40+IL-21 and IL-4 [100, 101].

### 5.2. Autoimmunity

Autoimmune manifestations are common (10-30%) among PID patients [102]. Autoimmunity almost is present in 21% to 42% of these patients [103, 104]. Physicians should consider a diagnosis of CVID in patients with autoimmune features in order to prevent diagnostic and therapeutic delays [105]. Autoimmune cytopenias (Idiopathic thrombocytopenic purpura (ITP), autoimmune hemolytic anemia (AIHA) and autoimmune neutropenia) are the most common and potentially severe manifestations [106, 107], while other autoimmune manifestations like rheumatologic autoimmune disorders are also observed in these patients [108-112].

Autoimmunity is the aberrant response of the immune system to self-antigens that occurs when self-tolerance is impaired. Several factors, which influence T-cell tolerance induction such as lower T cell and Treg numbers and aberrations in cytokine secretion, have been described as mechanisms leading to autoimmunity in CVID patients [67, 113]. Class switch recombination (CSR) and somatic hyper mutation (SHM) defects, increased levels of B cell-activating factor (BAFF), impaired Toll-like receptor (TLRs) and abnormalities in lymphoid cells subsets have been reported in CVID patients and are likely associated to the development of autoimmune manifestations [111, 114-116]. It is notable that despite the low serum level of immunoglobulins and poor specific antibody response in CVID
patients, autoantibodies are often found in a particular group of CVID cases [117]. Although
intriguing, these sometimes counter intuitive observations underscore the necessity of further
researches to reveal the underlying mechanisms leading to autoimmunity in CVID.

5.3. Lymphoproliferation and malignancy
An increased risk of malignancies has been reported in CVID patients [118]; however, the precise
incidence and mechanism of this association are still unclear. It has been estimated that the incidence
of malignancy among CVID patients is almost 2.5 % when the age at onset of symptoms is <16 years,
whereas this rate is almost 8.5 % among for patients diagnosed at an older age [118]. CVID patients
with polyclonal lymphadenopathy have an increased risk of lymphoid malignancies, being the overall
risk for lymphoid malignancies (mostly extra-nodal non-Hodgkin’s B cell lymphoma) 2-10% for
CVID patients [9, 107].

Additionally CVID patients are prone to develop malignant gastric cancers [103, 119]. Impaired
immunity to carcinogenic pathogens such as Epstein-Barr virus and H pylori potentially contributes to
this. Ten-Fold gastric cancer risk has been reported in CVID [120], but this ratio has been decreased
recently [9] potentially due to increased use of antibiotics at least partially treating and controlling H
pylori. This reduces the rates of chronic atrophic gastritis and metaplasia in the gastric body, which
are the most important predisposing factors for gastric adenocacinoma in CVID patients [121, 122].

5.4. Enteropathy
A selected group of CVID patients suffers from complex gastrointestinal disorders refractory to
conventional treatments resulting in significant weight loss and malnutrition that in some cases require
long-term parenteral feeding [107, 123]. Histopathology in tissue samples obtained from patients with
small bowel disease commonly shows villous atrophy and inflammatory lymphocytic infiltrates [95,
123]. Besides the obvious nutritional, gastrointestinal and infectious complications which are shared
with many other PID patients, CVID patients with this specific enteropathy phenotype are prone to
develop complications due to changes of bone mineral density, granulomatous disease, lymphopenia
and exhibit an overall higher mortality rate [124, 125].

5.5. Asthma and allergic diseases
Patients with CVID and IgA deficiency are predisposed to develop atopic conditions probably due to
mucosal immune defects as well as immune dysregulation with skewing to towards a TH2 phenotype
[126]. However, the data regarding the prevalence of atopic diseases in CVID is incomplete and only a
few reports are available on the incidence of asthma and allergic disorders in CVID patients [126, 127]. According to the currently available data, the prevalence of allergic disorders (including asthma, allergic rhinitis, atopic dermatitis, allergic eczema, food allergy, urticaria, allergic conjunctivitis and drug allergy) ranges from 12-42% in the different CVID cohorts [126, 128]. This discrepancy could be explained by factors such as sample sizes, underlying genetic defects, and ethnic composition. It seems that immune dysregulation, major histocompatibility complex haplotypes, impaired IgA response to luminal allergen challenge, high IgE level (because of a compensatory mechanism for decreasing other antibody isotypes) and persistent pulmonary infections are major causes of asthma and other atopic diseases in CVID patients [126]. Similar to other complications, a high suspicion and prompt diagnosis of atopic disorders are important when caring for CVID patients as this likely will impact their management and quality of life.

5.6. Other clinical findings
Neurologic and liver diseases are less commonly reported in CVID patients [129]. Infectious etiologies of the nervous system have described in 43 cases and represent the biggest class of neurologic dysfunction [130, 131], followed by autoimmune/inflammatory myelitis [132, 133]. Neuroendocrine alterations [134, 135] and nutritional deficiencies (vitamin E and B12 deficiency) have been reported but are in general uncommon [136, 137]. Almost 10% of CVID patients manifest significant liver with an increase in alkaline phosphatase level [138]. Primary biliary cholangitis, granulomatous liver disease [139] and idiopathic non-cirrhotic portal hypertension (including nodular regenerative hyperplasia) are the most commonly observed manifestations in CVID patients [138, 140].

6. Diagnosis
CVID patients have a broad phenotype with heterogeneous clinical and immunological features. This contributes to diagnostic difficulties and delays. Maintaining a high index of suspicion for CVID in patients with complex clinical manifestations and following the established diagnostic criteria is warranted in order to establish a timely and precise diagnosis.

Generally, hypogammaglobulinemia and recurrent infections are the hallmarks of a CVID diagnosis [141]. Diagnostic criteria have been proposed by the ESID (European society of immune deficiencies) in 1999 and redefined in the later years using both laboratory findings and clinical symptoms [142]. The newest ESID criteria for CVID diagnosis are illustrated below:
At least one of the following:
- increased susceptibility to infection
- autoimmune manifestations
- granulomatous disease
- unexplained polyclonal lymphoproliferation
- affected family member with antibody deficiency
- AND marked decrease of IgG and marked decrease of IgA with or without low IgM
- AND at least one of the following:
- poor antibody response to vaccines (and/or absent isohaemagglutinins);
- low switched memory B cells (<70% of age-related normal value)
- AND secondary causes of hypogammaglobulinaemia have been excluded
- AND diagnosis is established after the 4th year of life (but symptoms may be present before)
- AND no evidence of profound T-cell deficiency, defined as 2 out of the following (y=year of life):
  - CD4 numbers/microliter: 2-6y <300, 6-12y <250, >12y <200
  - % naive CD4: 2-6y <25%, 6-16y <20%, >16y <10%
  - T cell proliferation absent

Practically, to differentiate a patient with CVID from children with other risk factors predisposing them to recurrent infections taking a family history and physical examination are helpful. Based on history and physical examination laboratory investigation should be performed, but must be supplemented by immune function testing [143]. Every screening evaluation for CVID must include a physical examination of the patient. However, clinical symptoms can vary from patient to patient, even among affected family members with identical mutations of the same gene [144-146]. After physical examination and history-taking, the physician should be suspected of PID and a set of primary para-clinical evaluation should be taken for the patient (Figure1).

Laboratory and para-clinical evaluations

Initial evaluation: The most useful first-line immunological investigations that target CVID and majority of common CVID-like entities includes testing for a complete blood count with differential (CBC-Diff), lymphocyte subset analysis, and measurement of serum immunoglobulin, these tests can identify children who need further testing and referral to a subspecialist [147]. Patients with CVID have low serum Ig levels and/or decreased response to vaccination [148]. Serum Ig levels vary with age, so age-specific cutoffs should be used when performing Ig assay. Protein loss should also consider in patients with low
Ig in serum, therefore, in suspected individual serum albumin level should be checked because low albumin suggests protein loss through the kidney or malabsorption of the protein in the bowel. IgG antibody titers to vaccine antigens can be checked to determine specific antibody response. Both protein and polysaccharide antigens usually use for evaluation [149]. A vaccine with protein antigens can be checked at all ages; while immunization with polysaccharide antigens can be evaluated if the patient is 2 years or older. If the physician is using a conjugated pneumococcal vaccine, IgG antibody titers against specific serotype are needed to be measured [148].

Secondary evaluations: In addition to the global assessment of immune development through measurement of nonspecific features, such as serum immunoglobulin levels and leukocyte and lymphocyte subpopulations, evaluation of the specific immune response is essential. When a patient had abnormality in screening tests related to humoral immunity, following advanced tests are recommended to perform: IgG subclass analysis, flow cytometry to enumerate B-cell subsets (e.g., naive and switched memory cells), in vitro immunoglobulin production in response to mitogens or other stimuli, specific antibody response to immunization with φX174 [150].

Tertiary evaluations: Genetic testing plays a vital role in the diagnosis of CVID. There are several tools including molecular and cytogenetical tests that we can pick to exploit in genetic testing in CVID. Currently, chromosomal microarray (CMA) is useful for copy number variants (CNV) detection when no disease causing variants have detected after exome sequencing or when CNV prediction data indicated the presence of a relevant CNV [151]. CNV analysis has been performed in CVID to recognize the genetic basis of this heterogeneous immunological disorder [152, 153].

The ‘gold standard’ method of mutation screening is DNA sequencing using the dideoxy chain termination method that is considered the 1st generation of sequencing. This method is highly accurate and can detect point mutations, and some deletions and duplications. Sanger sequencing is applied in the diagnosis of circumstances that one gene or small set of genes is most likely causative. Sanger sequencing is a necessary tool for validation of variants detected using high throughput sequencing and also a reliable and cost effective method for evaluating family members of an affected patient for known mutations (segregation analysis) [4].

Next-generation sequencing (NGS) has a great implementation on clinical diagnostics for many genetic diseases. Currently, three NGS technologies including targeted sequencing (TGS), whole exome sequencing (WES) and whole genome sequencing (WGS) are in molecular service of diagnosis and
research in CVID and its new entities. The most focused NGS approach is the TGS, which sequences customizable sets of genetic targets covering a known group of disease-causing genes. The utility of TGS is inherently limited because this approach is restricted to a set list of target genes. However, in the situations that we have a list of multiple possible genes, TGP is reasonable. WES is a focused technology that sequences only the protein-coding regions, which contain around 85% of disease-causing mutations and cover 90 to 95% of exomes [154]. As the most of known monogenic causes of CVID-like phenotypes were identified in individual patients, rather than large families [155], it may not be rational to use TGS in patients with complex phenotypes that the novel causative gene is possible. The most comprehensive NGS technique is WGS, which covers the entire span of human DNA, including both coding and noncoding regions. Currently, WGS is not widely used clinically in CVID and it is the final choice where previous methods like TGP or WES cannot find causative variation [156]. Finally, a genetic investigation of patients can be helpful for a better prognosis, better defining the follow up and also genetic concealing.

7. Classification

CVID patients manifest various clinical and immunological features, thus the classification of these patients seems to be imperative. To date, several classifications have been proposed based on clinical manifestations and laboratory data in CVID patients. The Freiburg classification was established in 2002 by Warnatz et al. based on switched memory and CD21low B cells. Later, Piqueras et al. suggested the Paris classification based on memory B cells populations only. Wehr et al. established a comprehensive classification in 2008 that is now known as EUROclass [41]. Recently, a fourth classification based on B cell subset abnormalities has been defined by Driessen et al. [8].

7.1. Freiburg classification

The Freiburg classification distinguishes CVID patients based on the percentages of switched memory and CD21low B cells. Indeed, this classification discriminates patients with disturbed germinal center reactions and defective early peripheral B cell differentiation by analyzing CD21 expression. In this classification, patients are categorized into 2 groups based on the expression of IgM, IgD, CD27 (as a marker for Memory B cells) and CD21. Group I with severely reduced class-switched memory CD27IgMIgDIgD B cells (switched memory B-cells < 0.4%) and group II with normal switched memory B-cells > 0.4%. Furthermore, type I patients are subdivided into group Ia with highly expanded CD21low B cells (CD21lowB-cells >20%) and group Ib with percentages of CD21lowB-cells <20%. Splenomegaly and/or autoimmune manifestations are characteristic features of group Ia [8, 36, 40].

7.2. Paris Classification
The percentages of switched memory B cells and total CD27+ B cells are used for this classification. Patients with a reduced percentage of CD27+B cells < 11% represent the group MB0, while patients with decreased class-switched memory B-cells < 8% and increased total CD27+ B cell >11% of B-cells are classified as MB1. Group MB2 refers to patients who not fulfill the MB0 or MB1 criteria. According to this classification, the incidence of splenomegaly, granulomatous disease or lymphoid proliferation was higher in patients of group MB0 when compared to MB1, whereas the incidence of autoimmunity was higher in patients categorized as MB0 and MB1 groups [40].

One major difference between the Paris and the Freiburg classification is that the Paris classification calculates the class switched memory B cells by the percentage of total B cells, whilst for the Freiburg classification the memory B cells are calculated by the percentage of all peripheral blood lymphocytes. Both classifications show a correlation between the severity of disease and the proportion of switched memory B cells [41].

### 7.3. EUROclass Classification

EUROclass is a multicenter European trial that classifies patients based on their percentage of CD19 B cells. Thus, patients with ≤1% CD19 B cells are designated as group B− and those with a higher number of B cells (>1%) are designated as group B+. Group B+ is then divided into smB− with ≤2% switched memory B cells and smB+ with >2% of switched memory B cells groups. The smB− patients group is further divided into group smB'Trhi with ≥9% transitional B cells (CD21int CD38++ IgM++) and group smB'Trnorm with <9% transitional B cells. In addition, EUROclass distinguishes patients based on the expansion of CD21low B cells. Patients with ≥10% CD21low B cells are known as group CD21low and those with <10% are categorized as CD21norm allowing overlap between patients with the expansion of CD21low and transitional B cells. This classification is currently the most suitable in order to predict complications such as granulomatous disease, lymphadenopathy, and splenomegaly in CVID patients. Based on this classification, CVID patients with a severe decreased smB are associated with a higher risk for splenomegaly and granulomatous disease. Moreover, CVID patients with splenomegaly are associated with an expansion of CD21low B cells while lymphadenopathy is significantly linked with transitional B-cell expansion [41]. In a recent study, we also confirmed an association between the presence of smB’CD21low and splenomegaly in CVID patients [8].

### 7.4. B cell pattern classification

According to this classification, patients are divided into five distinct groups based on their B cells subsets. Patients in group 1, show decreased numbers of transitional B-cells along with a reduction of memory B-cells. Group 2 patients have a reduced number of transitional B-cells, naive, marginal zone-
like and memory B cells. Patients with a reduction of both marginal zone-like and memory B-cells are classified in group 3, whilst in group 4, patients show only decreased memory B-cells. Finally, patients in group 5 show normal marginal zone-like and memory B-cells in combination with a reduction in plasmablasts count.

7.5. Other immunologic classification

Light chains are physiologically expressed in slight excess resulting in free light chains detectable in serum (sFLC). Detection of sFLCs in CVID by Hanitsch et al. [157] showed that 37% had normal kappa and lambda chains (κ+/λ+), 12% reduced kappa chains (κ−/λ+), 5% reduced lambda chains (κ+/λ−), and in 46% reduction of both chains (κ−/λ−). Of note, there was a clinical correlation between κ+/λ− sFLCs phenotype and the development of recurrent pneumonia, bronchiectasis and lymphoproliferative disease. Moreover, there is a classification based on clinical manifestation proposed by Chapel H et al. in 2008. According to this classification, patients are divided into 5 distinct clinical phenotypes including no complications, autoimmunity, polyclonal lymphocytic infiltration, enteropathy, and lymphoid malignancy [103].

8. Management

As mentioned above, hypogammaglobulinemia is the hallmark of CVID and Ig replacement therapy is the most important therapeutic intervention [158]. Indeed, Ig replacement therapy decreases the rates of recurrent and severe infections and consequent hospitalizations [158]. Some patients with comorbidities, protein losing conditions, as well as pregnant patients, may require dose adjustment [159, 160]. In addition, prophylactic and therapeutic antibiotics and complementary vaccinations with inactive antigens are recommended in these patients [158]. Vaccination in a subgroup of CVID patients with post-germinal center B-cell pattern is effective both in boosting humoral and cellular immunity [161]. As hepatic and renal dysfunctions are not uncommon in CVID patients, therapeutic modalities should be adjusted when necessary [162].

Furthermore, since CVID patients are prone to non-infectious complications such as autoimmunity, the caring physician should continuously monitor for the occurrence of these manifestations. In addition, specific treatment of autoimmune complications with immune modulation might be indicated. Herein, it should be noted that treating physician may consider immunosuppressive drugs such as Abatacept, Infliximab and Rituximab in the treatment of autoimmune manifestations of CVID patients with CTLA-4 and LRBA mutations or mTOR inhibitors especially in patients with PI3K signaling defect [163].
HSCT a potentially curative treatment approach has been performed in some cases of CVID with mixed results. Currently, HSCT only being advised in extremely severe CVID cases mostly associated with cellular immune defects and therapy resistant autoimmunity because of high mortality in spite of being beneficial in most surviving patients. However, it may become a potential treatment for specific CVID genetically characterized forms [10].

9. Conclusion
In this article, we reviewed the epidemiology, clinical manifestations, pathogenesis, diagnosis, classifications and management of CVID patients. This review provides a comprehensive overview of CVID but it is noteworthy that to date many highly relevant questions remain unanswered. This is likely due to the extreme heterogeneity of this disorder. The establishment of generally accepted diagnostic criteria, as well as the introduction of patients according to these criteria in international registries steps mandatory first steps in order to design and perform meaningful studies. The identification of patients with monogenetic defects within the CVID cohorts has helped to increase our understanding of the pathogenesis of this complex disorder and might enable us to offer targeted treatment strategies beyond Ig replacement in the future.

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Conflicts of Interest
The authors declare that they have no conflicts of interest.
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Table 1. Surface molecular defects in CVID patients

<table>
<thead>
<tr>
<th>Name of Genes</th>
<th>Functions</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>TACI (encoded by TNFRSF13B)</td>
<td>Function: Regulation of BCR, TLR7, and TLR9 function</td>
<td>[18, 20, 164]</td>
</tr>
<tr>
<td></td>
<td>Effect of deficiency: B cell activation defect and also autoimmunity manifestation</td>
<td></td>
</tr>
<tr>
<td>BAFF-R (encoded by TNFRSF13C)</td>
<td>Function: Regulation of B cell survival and maturation</td>
<td>[43]</td>
</tr>
<tr>
<td></td>
<td>Effect of deficiency: Low peripheral B-cell numbers, Increased transitional B cells, decreased antibody responses to polysaccharide vaccine</td>
<td></td>
</tr>
<tr>
<td>TWEAK (encoded by TNFRSF12)</td>
<td>Function: Promotion of endothelial cells proliferation and polarization of immune responses to TH1 adaptive responses</td>
<td>[165]</td>
</tr>
<tr>
<td></td>
<td>Effect of deficiency: Inhibition of B-cell survival and proliferation, Inhibition of Ig class switching by down regulation of the noncanonical BAFF-induced NF-κB pathway</td>
<td></td>
</tr>
<tr>
<td>CD27 (encoded by TNFRSF7)</td>
<td>Function: Participation in T, B and NK-cell function</td>
<td>[166, 167]</td>
</tr>
<tr>
<td></td>
<td>Effect of deficiency: Hypogammaglobulinemia, abnormal T-cell dependent B-cell response, disturbed T-cell function, absent memory B cell</td>
<td></td>
</tr>
<tr>
<td>CD19 complex (CD19, CD81 and CD21)</td>
<td>Function: Attenuation of B cell activation threshold and thus signaling enhancement as a result of co-ligation of this complex by B cell receptor.</td>
<td>[168-172]</td>
</tr>
<tr>
<td></td>
<td>Effect of deficiency: Impaired BCR/co-receptor complex signaling, defective somatic hypermutation and CSR, reduced memory B cells and plasma cells, are seen in both CD19 and CD81 deficient patients. Hypogammaglobulinemia, reduced number of memory B cells and increased naïve mature B cells have been observed in CD21 deficient patients.</td>
<td></td>
</tr>
<tr>
<td>IL21R and its ligand (IL21)</td>
<td>Function: IL21-IL21R interaction is required for germinal center formation, proliferation and class switch recombination of B cells, plasma cell differentiation and eventually immunoglobulin production.</td>
<td>[173-176]</td>
</tr>
<tr>
<td></td>
<td>Effect of deficiency: defects in class switch recombination, variable dysfunctions of NK cell cytotoxicity, decreased T cell proliferation, reduced numbers of circulating, marginal zone-like and class-switched memory B cells, increased number of transitional ones, increased IgE levels and reduced IgG levels.</td>
<td></td>
</tr>
<tr>
<td>ICOS</td>
<td>Function: Regulation of terminal B cell differentiation in germinal centers, T cell tolerance and also effector T cell responses.</td>
<td>[177]</td>
</tr>
<tr>
<td></td>
<td>Effect of deficiency: Severe reduction in B lymphocyte counts, low levels of serum concentrations of IgM and switched Ig isotype, lack of the expression of maturation and memory marker CD27 on B cells</td>
<td></td>
</tr>
<tr>
<td>CD20 (MS4A1)</td>
<td>Function: Regulation of Ca2+ transport across the plasma membrane.</td>
<td>[178]</td>
</tr>
<tr>
<td></td>
<td>Effect of deficiency: Severe reduction in switched memory B cells, decreased IgG level with relatively increased IgM and weak responses against polysaccharides after vaccination, reduction in the frequency of somatic hypermutation in IgG heavy chain</td>
<td></td>
</tr>
<tr>
<td>FCγRIIa</td>
<td>Function: Recognition of FC region of immunoglobulin G</td>
<td>[179, 180]</td>
</tr>
<tr>
<td></td>
<td>Effect of deficiency: Increased sensitivity of neutrophils to immune complexes, anaphylactoid reactions to immunoglobulin infusions, suppress signaling cascade in B cells due to MAPK phosphorylation</td>
<td></td>
</tr>
<tr>
<td>CTLA-4</td>
<td>Function: Suppression of immune responses by negative signaling and therefore preventing excessive T cell activation</td>
<td>[181, 182]</td>
</tr>
<tr>
<td></td>
<td>Effect of deficiency: Impaired function of Treg cells, reduced circulating B cells, increased autoreactive CD21low</td>
<td></td>
</tr>
</tbody>
</table>


doi: 10.18176/jiaci.0388
Table 2. Cytosolic defects in CVID patients

<table>
<thead>
<tr>
<th>Name of Genes</th>
<th>Functions</th>
<th>Ref</th>
</tr>
</thead>
</table>
| **PKCδ**     | Function: Involved in BCR mediated signaling and is participated in the regulation of cellular process including proliferation, differentiation, apoptosis and tolerance  
Effect of deficiency: Decrease of CD19 B cells, low numbers of memory B cells, as well as increased numbers of CD21<sup>low</sup> B cells | [183-185] |
| **PLCγ2**    | Function: Participation in B cell receptor signaling  
Effect of deficiency: Defective calcium flux and phosphorylation of ERK in response to IgM crosslinking, abnormal class switch recombination and receptor editing and also antibody deficiency | [186, 187] |
| **PI3K (PI3KR1, PI3LCD)** | Function: B and T cell homoeostasis  
Effect of deficiency: Expanded CD8 T cells, agammaglobulinemia, increased the frequency of transitional B cells, decreased numbers of naïve CD4 and CD8 T cells and increased numbers of CD8 effector/memory T cell, normal or often increased serum IgM kevels, increased T cell activation-induced cell death | [188-191] |
| **BLK**      | Function: Member of the Src kinase family which is involved in BCR signaling  
Effect of deficiency: Diminished B-cell proliferation and T-cell help and subsequently reduced numbers of class-switched memory B-cells and defective production of high affinity antibody | [192] |
| **IP3**      | Function: Induction of calcium mobilization to the cytosol, resulting in elevation of the intracellular Ca<sup>2+</sup> that is necessary for the induction of the IL-2 gene  
Effect of deficiency: Defect in TCR signaling | [193, 194] |
| **LCK**      | Function: A tyrosine kinase associated with the cytoplasmic tails of CD4 and CD8 in T-cells and is involved in the maturation, activation and differentiation of T cells  
Effect of deficiency: Normal T cell number, reduced T regulatory cells, impaired TCR signaling and restricted T cell repertoire | [195] |
| **Vav1**     | Function: Required for T cell activation and T helper polarization to Th2 subsets  
Effect of deficiency: T cell dysfunction | [196, 197] |
| **Rac2**     | Function: A member of the Rho family of GTPase that are crucial regulators of cell signaling and actin cytoskeleton  
Effect of deficiency: Reduced chemotaxis activity, reduced numbers of neutrophil granules as well as morphological changes of the secondary granules, defects in B and T cells development | [198-200] |
| **ZAP70**    | Function: A member of the Syk family of tyrosine kinases that play an important role in T cell activation  
Effect of deficiency: Impaired T cell function due to defective recruitment and activation of ZAP70 | [201] |
| **LRBA**     | Function: A cytosolic protein participate in multiple cellular functions such as vesicular trafficking, signal transduction, cytoskeleton assembly, transcriptional regulation, autophagy and apoptosis  
Effect of deficiency: Hypogammaglobulinemia and reduced switched memory B cells as well as different clinical manifestation such as autoimmunity, enteropathy, and recurrent respiratory infections | [202-204] |
| **ERK**      | Function: A serine/threonine kinase that phosphorylates various substrates and plays important roles in cell proliferation, differentiation, migration and survival  
Effect of deficiency: Dysregulation of BCR induced ERK activation in naïve and IgM memory B cells, blocking of BCR endocytosis and B cell dysfunction especially in CVID patients with CD21<sup>low</sup> phenotype | [205] |
| **CARMA1/CARD11** | Function: T and B cell activation through NF-κB activation after TCR and BCR cross-linking  
Effect of deficiency: Impaired B cell differentiation and T cell proliferation, reduced number of T regulatory cells and increased number of | [206, 207] |
|---|---|
| **Bob1** | **Function:** A B-cell–specific transcriptional co-activator that stimulates transcription in selected immunoglobulin genes  
**Effect of deficiency:** Decreased B cell production and activation, impaired germinal center formation and reduced class switched immunoglobulins | [207, 208] |
| **TLRs** | **TLR9** | **Function:** Recognition of DNA-containing CpG motifs derived from microbes and play key roles in the activation of immune responses  
**Effect of deficiency:** Normal number of B cells but decreased circulating memory and switched memory CD27⁺B cells, decreased plasma cells and increased transitional B cells | [73, 181, 209] |
| | **TLR7** | **Function:** Recognition of single-stranded RNA derived from microbes  
**Effect of deficiency:** Defective B cells proliferation, lack cytokine production, impaired IgG and IgA production in both naïve and memory B cells. These defects are also seen in pDCs as it is demonstrated that these cells fail to produce IFN-α in response to TLR ligands |  |
Table 3. Nucleus defects in CVID patients

<table>
<thead>
<tr>
<th>Name of Genes</th>
<th>Functions</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>NFKB (NFKB1, NFKB2)</strong></td>
<td>Function: NFkB signaling play important roles in B cell maturation, differentiation, survival, class switching and tolerance to self-antigens</td>
<td>[210-212]</td>
</tr>
<tr>
<td></td>
<td>Effect of deficiency: Defective development or maintenance of Tfh cells, Impaired T cell and NK cell functions, reduced switched memory B cell counts, (pan) hypogammaglobulinemia</td>
<td></td>
</tr>
<tr>
<td><strong>IKZF1</strong></td>
<td>Function: A member of a family of hematopoietic zinc-finger transcription factors that play key roles in B cell lymphopoiesis and function.</td>
<td>[213]</td>
</tr>
<tr>
<td></td>
<td>Effect of deficiency: Panhypogammaglobulinemia, low B cell number with a progressive loss of serum immunoglobulins and B cells</td>
<td></td>
</tr>
<tr>
<td><strong>STAT1</strong></td>
<td>Function: A member of the transcription protein family important in many biological processes.</td>
<td>[214]</td>
</tr>
<tr>
<td></td>
<td>Effect of deficiency: Hypogammaglobulinemia, reduced switched memory and plasma cells, increased proportion of naïve, CD21low and transitional B cells as well as reduced numbers of IL-17-producing CD4+ T cells and T regulatory cells</td>
<td></td>
</tr>
<tr>
<td><strong>IRF2BP2 (Interferon regulatory factor 2 binding protein 2)</strong></td>
<td>Function: A negative regulator of the NFAT transcription factor, play a role in the differentiation and/or survival of memory B cells and plasmablasts</td>
<td>[215]</td>
</tr>
<tr>
<td></td>
<td>Effect of deficiency: Relative decrease in switched memory B-cells, undetectable IgG2, absent IgA and low IgM, decreased the formation of B-cell plasmablasts</td>
<td></td>
</tr>
<tr>
<td><strong>NEIL1</strong></td>
<td>Function: DNA glycosylases that participate in base excision repair and B cells development and function</td>
<td>[216]</td>
</tr>
<tr>
<td></td>
<td>Effect of deficiency: Increased naïve memory B-cells (IgD CD27+), reduced count of marginal zone B-cells (IgD CD27+) and an almost complete absence of class switched memory B-cells and low level of immunoglobulins</td>
<td></td>
</tr>
<tr>
<td><strong>SEC61A1</strong></td>
<td>Function: The major subunit of the Sec61 complex, which is the 57 main polypeptide-conducting channels in the endoplasmic reticulum membrane which is strongly induced during plasma cell differentiation.</td>
<td>[217]</td>
</tr>
<tr>
<td></td>
<td>Effect of deficiency: Impaired plasma cell homeostasis without interfering with B cell development, activation, and memory formation</td>
<td></td>
</tr>
<tr>
<td><strong>CD70</strong></td>
<td>Function: Participation in T cell expansion and survival, germinal center formation, B cell activation and antibody production, and NK cell function</td>
<td>[216]</td>
</tr>
<tr>
<td></td>
<td>Effect of deficiency: Increased susceptibility to EBV-induced disease as well as impairments in T and B cell differentiation, hypogammaglobulinemia, poor Ab responses to vaccinations, and/or a reduced percentage of switched memory B cells</td>
<td></td>
</tr>
<tr>
<td><strong>ATP6AP1</strong></td>
<td>Function: This gene encodes the accessory protein Ac45 of the V-ATPase</td>
<td>[218]</td>
</tr>
<tr>
<td></td>
<td>Effect of deficiency: Hypogammaglobulinemia, problem in B-cell differentiation</td>
<td></td>
</tr>
<tr>
<td><strong>TTC37</strong></td>
<td>Function: This gene encodes members of the human Ski complex, which functions in exosomal RNA degradation</td>
<td>[219]</td>
</tr>
<tr>
<td></td>
<td>Effect of deficiency: Specific antibody deficiency with impairment of humoral memory</td>
<td></td>
</tr>
<tr>
<td><strong>TRNT1</strong></td>
<td>Function: A template-independent RNA polymerase essential for maturation of both nuclear and mitochondrial transfer RNAs</td>
<td>[220]</td>
</tr>
<tr>
<td></td>
<td>Effect of deficiency: B-cell immunodeficiency</td>
<td></td>
</tr>
<tr>
<td><strong>PTEN</strong></td>
<td>Function: Downregulation of AKT signaling in the mTOR pathway which is critical for cell survival, proliferation, growth, and metabolism</td>
<td>[221]</td>
</tr>
<tr>
<td></td>
<td>Effect of deficiency: Hypogammaglobulinemia, reduced numbers of memory B cells and class-switched memory (CD27+IgM1gD1) B cells</td>
<td></td>
</tr>
</tbody>
</table>

NF-κB: nuclear factor kappa-light-chain-enhancer of activated B cells, Tfh: T follicular helper cells, NFAT: nuclear factor of activated T cells
Table 4. Abnormalities and Functions of different immune cells in CVID

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Subsets</th>
<th>Increased/Decreased</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>B cell</strong></td>
<td>Transitional B cells</td>
<td>Increased</td>
<td>[42]</td>
</tr>
<tr>
<td></td>
<td>CD21&lt;sub&gt;low&lt;/sub&gt; B cells</td>
<td>Increased</td>
<td>[43]</td>
</tr>
<tr>
<td></td>
<td>IgM memory B cells (CD19&lt;sup&gt;+&lt;/sup&gt;/CD27&lt;sup&gt;+&lt;/sup&gt;)</td>
<td>Decreased</td>
<td>[34]</td>
</tr>
<tr>
<td></td>
<td>Class switched memory B cells (CD19&lt;sup&gt;+&lt;/sup&gt;/CD27&lt;sup&gt;+&lt;/sup&gt;/IgD&lt;sup&gt;-&lt;/sup&gt;/IgM&lt;sup&gt;-&lt;/sup&gt;)</td>
<td>Decreased</td>
<td>[39]</td>
</tr>
<tr>
<td></td>
<td>Plasma cells</td>
<td>Decreased</td>
<td>[40, 222]</td>
</tr>
<tr>
<td><strong>T cell</strong></td>
<td>Naïve CD4&lt;sup&gt;+&lt;/sup&gt; T cells</td>
<td>Decreased</td>
<td>[46, 48]</td>
</tr>
<tr>
<td></td>
<td>Effector memory CD4&lt;sup&gt;+&lt;/sup&gt; T cells</td>
<td>Increased (in all 3 groups Ia/Ib/II)</td>
<td>[46]</td>
</tr>
<tr>
<td></td>
<td>Central memory CD4&lt;sup&gt;+&lt;/sup&gt; T cells</td>
<td>Increased (in all 3 groups Ia/Ib/II)</td>
<td>[46]</td>
</tr>
<tr>
<td></td>
<td>Activated CD4&lt;sup&gt;+&lt;/sup&gt; T cells</td>
<td>Increased</td>
<td>[46]</td>
</tr>
<tr>
<td></td>
<td>Th1/Th2</td>
<td>Trend towards Th1 or Th2</td>
<td>[50-52, 223]</td>
</tr>
<tr>
<td></td>
<td>Th17 cells</td>
<td>Decreased</td>
<td>[53]</td>
</tr>
<tr>
<td></td>
<td>Follicular T helpers (CD4&lt;sup&gt;+&lt;/sup&gt;/CXCR5&lt;sup&gt;+&lt;/sup&gt;) cells</td>
<td>Increased/ Decreased</td>
<td>[55, 56, 58]</td>
</tr>
<tr>
<td></td>
<td>T regulatory cells</td>
<td>No difference between the patients and healthy control/ Decreased</td>
<td>[48, 60, 61, 67]</td>
</tr>
<tr>
<td></td>
<td>Naïve CD8&lt;sup&gt;+&lt;/sup&gt; T cells</td>
<td>Decreased</td>
<td>[48]</td>
</tr>
<tr>
<td></td>
<td>Effector memory CD8&lt;sup&gt;+&lt;/sup&gt; T cell</td>
<td>Increased (in all 3 groups Ia/Ib/II)</td>
<td>[46, 48]</td>
</tr>
<tr>
<td></td>
<td>Central memory CD8&lt;sup&gt;+&lt;/sup&gt; T cell</td>
<td>Increased (in group I)</td>
<td>[46]</td>
</tr>
<tr>
<td></td>
<td>Activated CD8&lt;sup&gt;+&lt;/sup&gt; T cells</td>
<td>Increased</td>
<td>[66, 67]</td>
</tr>
<tr>
<td></td>
<td>γδ&lt;sup&gt;+&lt;/sup&gt; T cells</td>
<td>Increased</td>
<td>[70, 224, 225]</td>
</tr>
<tr>
<td><strong>Dendritic Cells</strong></td>
<td>DCs cells</td>
<td>Decreased</td>
<td>[74, 226, 227]</td>
</tr>
<tr>
<td><strong>Monocytes/Macrophages</strong></td>
<td>IL-12&lt;sup&gt;-&lt;/sup&gt; monocytes</td>
<td>Increased</td>
<td>[77]</td>
</tr>
<tr>
<td><strong>ILC</strong></td>
<td>IL-17&lt;sup&gt;+&lt;/sup&gt;CD127&lt;sup&gt;+&lt;/sup&gt;/Thy1&lt;sup&gt;+&lt;/sup&gt; Lin&lt;sup&gt;+&lt;/sup&gt; cells</td>
<td>Decreased</td>
<td>[54]</td>
</tr>
<tr>
<td></td>
<td>Lin CD127&lt;sup&gt;+&lt;/sup&gt; cells producing IFN-γ, IL-17A and IL-22</td>
<td>Increased</td>
<td>[228]</td>
</tr>
<tr>
<td></td>
<td>CD3&lt;sup&gt;-&lt;/sup&gt; CD16&lt;sup&gt;-&lt;/sup&gt; CD56&lt;sup&gt;-&lt;/sup&gt; CD28&lt;sup&gt;+&lt;/sup&gt; NK cells</td>
<td>Increased</td>
<td>[83]</td>
</tr>
<tr>
<td>CD3−CD16−CD56−CD28− NK cells</td>
<td>Decreased</td>
<td>[83]</td>
<td></td>
</tr>
<tr>
<td>-----------------------------</td>
<td>-----------</td>
<td>------</td>
<td></td>
</tr>
<tr>
<td><strong>NKT</strong></td>
<td>NKT cells</td>
<td>No difference between the patients and healthy control.</td>
<td>[85]</td>
</tr>
<tr>
<td><strong>iNKT</strong></td>
<td>iNKT cells</td>
<td>Decreased</td>
<td>[86]</td>
</tr>
</tbody>
</table>

*NKT: Natural killer T, iNKT: Invariant natural killer T*
Figure 1. General approach for the diagnosis of CVID. After physical examination and history-taking, the physician should evaluate patients suspected to primary immunodeficiencies in three steps of initial, secondary and tertiary evaluations for diagnosis of CVID patients.