

Common variable immunodeficiency: a new look at an old disease

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Primary immunodeficiencies comprise many diseases caused by genetic defects primarily affecting the immune system. About 150 such diseases have been identified with more than 120 associated genetic defects. Although primary immunodeficiencies are quite rare in incidence, the prevalence can range from one in 500 to one in 500 000 in the general population, depending on the diagnostic skills and medical resources available in different countries. Common variable immunodeficiency (CVID) is the primary immunodeficiency most commonly encountered in clinical practice, and appropriate diagnosis and management of patients will have a significant effect on morbidity and mortality as well as financial aspects of health care. Advances in diagnostic laboratory methods, including B-cell subset analysis and genetic testing, coupled with new insights into the molecular basis of immune dysfunction in some patients with CVID, have enabled advances in the clinical classification of this heterogeneous disease.

Introduction

Frequent sinopulmonary infections are a characteristic clinical presentation in many patients with primary immunodeficiencies. Antibody-related defects or humoral primary immunodeficiencies account for 65% of all primary immunodeficiencies, whereas defects in both the cellular and antibody compartments account for another 15% of the cases. Among the humoral primary immunodeficiencies, common variable immunodeficiency (CVID) generally comprises antibody deficiencies that present in either late childhood or, more typically, early to mid adulthood. The enormous heterogeneity in the clinical presentation of CVID poses a challenge to primary-care physicians who are most likely to encounter patients with the disorder due to their predisposition to infections. Delays in recognising CVID are common¹⁻³ in primary-care settings because of the pervasive misconceptions that primary immunodeficiencies are extremely rare, that the disorders are largely restricted to children, and that all patients are invariably moribund or seriously ill at the time of presentation.⁴

In 1953, Janeway and colleagues⁵ were the first to report CVID in a 39-year-old with recurrent sinopulmonary infections, bronchiectasis, and *Haemophilus influenzae* meningitis. Although the clinical entity of CVID has been known for over five decades, our understanding of the disease is far from complete. In this article, we focus on recent developments in this subject that have the potential to improve initial clinical and laboratory assessments of patients, enabling early appropriate diagnosis and management.

Epidemiology

Although selective IgA deficiency is the commonest primary immunodeficiency, most patients are asymptomatic,⁶ and CVID is the commonest clinically relevant primary immunodeficiency. In a European internet-based database, which included patients' and

research data on primary immunodeficiencies, 30% of the patients had CVID.⁷ Both sexes are affected equally, and the prevalence of CVID ranges from one per 50 000 to one per 200 000 with a reported incidence of one per 75 000 live births.⁸⁻¹⁰ Most patients have sporadic disease, but 10–25% have familial inheritance, typically with autosomal-dominant inheritance.¹¹⁻¹⁴

The age at presentation of CVID has a bimodal distribution. A few patients present in mid childhood but most present in early to mid adulthood, although some patients present even later. In one of the largest series of patients with CVID (n=248), which included patients aged 3–79 years, the mean age at onset of symptoms was 23 years for males and 28 years for females; while the mean ages of diagnosis were 29 years and 33 years, respectively.¹⁵

Search strategy and selection criteria

We searched Medline (1950–present), Embase (1988–present), Web of Science (1993–present), Cochrane Database of Systematic Reviews (from inception), Cochrane Central Register of Controlled Trials (from inception), and SCOPUS with the term “common variable immunodeficiency” for the years. We used the terms “immunologic deficiency syndromes”, “immune deficiency”, “agammaglobulinaemia”, “hypogammaglobulinaemia” in conjunction with keywords such as “CVID”, “common adj variable”, “primary”, “humoral”, “BAFF-R”, “TACI”, “ICOS”, “CD19”. We also used terms for the diseases and recurrent infections commonly associated with the syndrome: “sinusitis”, “rhinitis”, “pneumonia”, “asthma”. The searches were further limited to large epidemiological studies, cohort studies, clinical trials, meta-analysis, and areas specifically related to diagnosis (laboratory diagnosis, specificity and sensitivity, differential diagnosis, diagnostic accuracy, and clinical competence). We also searched the references of relevant articles.

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Clinical phenotype

Infections

CVID has a broad and heterogeneous phenotype (figure 1) that spans sinopulmonary and systemic bacterial infections and gastrointestinal complications.^{15,16} Most of 248 patients with CVID followed up for 1–25 years had recurrent bronchitis, sinusitis, otitis media, and pneumonia; while a few had viral hepatitis, severe Herpes zoster infection, and *Giardia* enteritis.¹⁵ The frequency of infectious presentation differed slightly in paediatric CVID populations, in which sinusitis is the commonest clinical presentation followed by otitis media and pneumonia.¹⁷

The recurrent infections of both the upper and the lower respiratory tract are over-represented for encapsulated (*H influenzae*, *Streptococcus pneumoniae*) or atypical (*Mycoplasma* spp) bacteria.^{8,15,18}

Autoimmune diseases

25% of patients with CVID have autoimmune events (figure 1). These events in CVID typify the underlying immune dysregulation in these patients, in whom specific checkpoints for autoreactivity during B-cell development¹⁹ either fail or are circumvented. This dysregulation leads to the generation of multiple

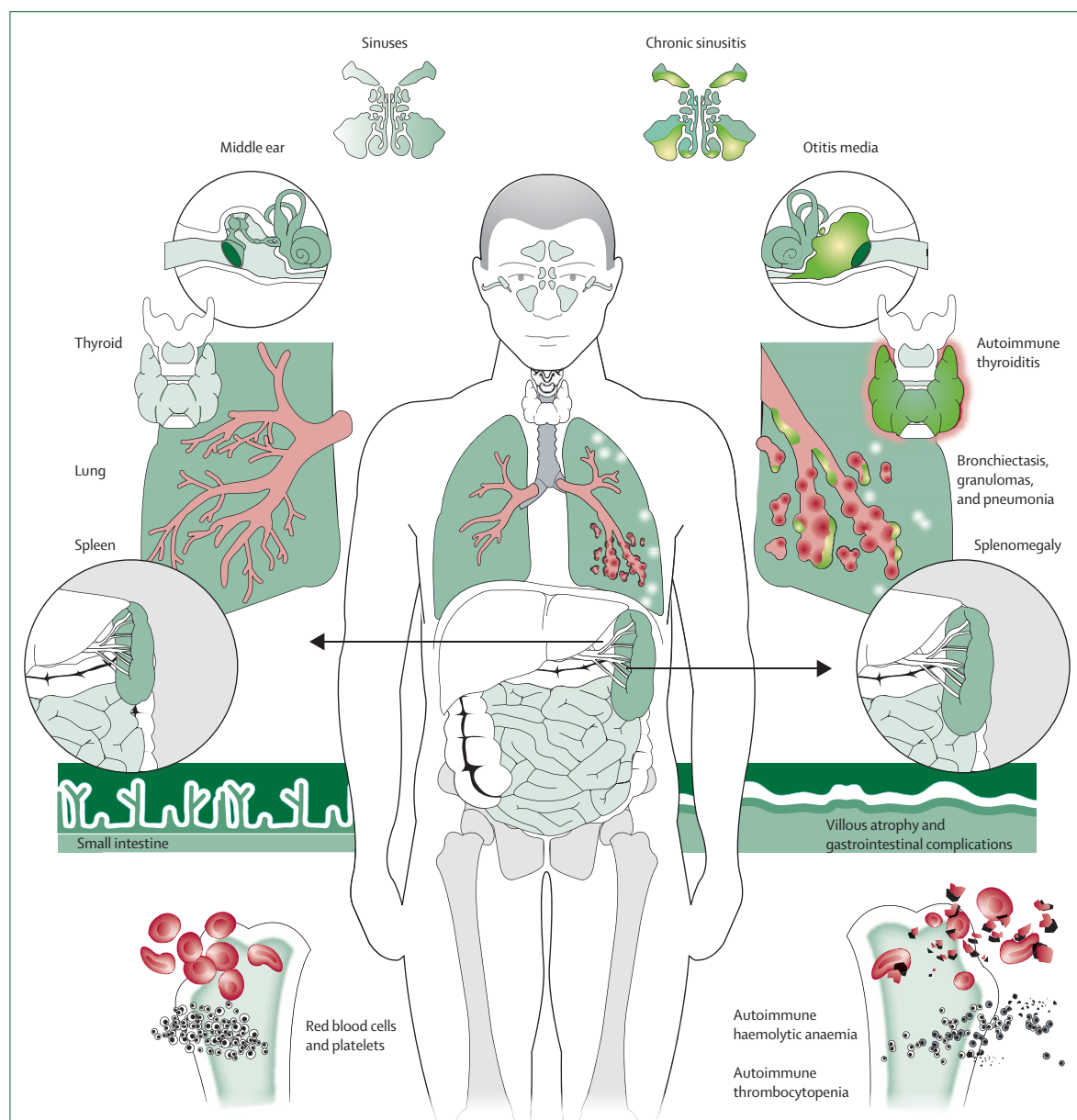


Figure 1: Organ systems involved in the pathogenesis of CVID

Left: healthy organs. Right: organ-system involvement. Patients also have increased risk of neoplasia, rheumatoid arthritis, vitiligo, and other autoimmune diseases. Reproduced with permission from the Mayo Foundation for Medical Education and Research.

	Laboratory features	Clinical features	Further testing
Selective IgA deficiency	Low titres of or absent IgA with normal IgG and IgM concentrations	Usually asymptomatic	Needed if symptomatic
Selective IgG subclass deficiency	Low titres of one or more of the IgG subclasses (G1, G2, G3, and G4); normal total IgG concentrations unless IgG1 is affected	Usually asymptomatic	Needed if symptomatic
X-linked agammaglobulinaemia	Modest to profound hypogammaglobulinaemia with low numbers or absence of peripheral-blood B cells	Recurrent infections	BTK protein expression by flow cytometry;* gene sequencing* if protein absent
X-linked lymphoproliferative syndrome	Hypogammaglobulinaemia, EBV infection is usually the trigger	Aberrant response to EBV, lymphoproliferative disease	SH2D1A protein expression by flow cytometry;* confirmation by SH2D1A gene sequencing*
Autosomal recessive agammaglobulinaemia	Profound hypogammaglobulinaemia with absent or very low numbers of peripheral B cells	Tends to manifest early in infancy or childhood, recurrent and severe infections	Gene sequencing of six genes identified (Igμ heavy chain, IgVκA2, CD79A, CD79B, λ5 surrogate light chain, BLNK)†
Hyper-IgM syndromes	Normal to high titres of IgM with low titres of IgG and IgA, very low numbers or absent class-switched memory B cells	Recurrent opportunistic sinopulmonary infections, patients with NEMO defects have hypohidrotic ectodermal dysplasia and susceptibility to recurrent mycobacterial infections	Protein expression by flow cytometry for CD40L on activated T cells and CD40 on B cells,* receptor-binding function for CD40L, confirmation by gene sequencing is available for the five genes implicated (CD40L, NEMO, CD40, AID, and UNG)*

Drugs, haematological malignancies, and other clinical phenotypes that can cause secondary hypogammaglobulinaemia are described in the text. *Tests available clinically in specialised reference laboratories. †Tests available only in the research setting. BTK=Bruton's tyrosine kinase. EBV=Epstein-Barr virus. CD40L=CD40 ligand. NEMO=NF-κB essential modulator. AID=activation-induced cytidine deaminase. UNG=uracil DNA glycosylase.

Table 1: Differential diagnosis for patients with suspected CVID (hypogammaglobulinaemia with recurrent infections)

autoantibodies against various antigenic targets.²⁰ Autoimmune thrombocytopenic purpura and autoimmune haemolytic anaemia are the most common autoimmune consequences, occurring in 5–8% of all patients with CVID.^{2,15,21} Some patients have onset of these disorders before the diagnosis of CVID. Therefore, CVID needs to be considered in the differential diagnosis of adult-onset autoimmune thrombocytopenic purpura and autoimmune haemolytic anaemia.²² A third of patients with CVID have splenomegaly.^{15,23} Other autoimmune disorders include the presence of anti-IgA antibodies,^{15,24} pernicious anaemia,^{20,25} and autoimmune thyroiditis.²⁶ Other less common autoimmune consequences of CVID include rheumatoid arthritis, vitiligo, and vasculitis.

Pulmonary complications

Chronic pulmonary complications including recurrent pneumonia are the primary cause of significant morbidity in patients with CVID (figure 1). Many patients have anomalies of lung parenchyma visible on chest radiographs and CT scans. The most common pulmonary CT findings include airway disease, ground-glass attenuation, nodules, and parenchymal opacification.²⁷ Pulmonary fibrosis and bronchiectasis might also present;²⁸ the latter is a common clinical finding in CVID and other immunodeficiencies.^{28–30} High-resolution CT is the best diagnostic tool for bronchiectasis.²⁸ As many as 50% of patients with CVID may have other pulmonary features presenting with an

obstructive lung phenotype, such as chronic bronchitis and asthma.³¹

Granulomatous disease

Multisystem granulomas are a well documented cause of increased morbidity and mortality in patients with CVID; the lungs are the most commonly affected site (figure 1), though other organs, such as liver, skin, spleen, and gastrointestinal tract can also be involved.^{15,32} Granulomatous disease in CVID might affect about 10–22% of patients^{1,33–35} and the mean age at onset is 18–34 years.^{33,34,36} Although granulomas are most common in adults, up to a third of paediatric patients with CVID can also have this complication.³² Presentation of granulomatous disease can precede a diagnosis of CVID³⁴ and the delay in recognition of CVID can affect prognosis. Histologically, the non-caseating granulomas in CVID resemble sarcoidosis. Angiotensin-converting enzyme concentrations are high in some patients with CVID.^{34,36} And preliminary evidence suggests that a human herpes virus 8 is a cause of systemic granulomatous disease and lymphoproliferative disorders in some patients with CVID.^{32,37} The prevalence of autoimmunity, particularly autoimmune haemolytic anaemia, is higher (>50%) in patients with CVID and granulomatous disease than in those without (~47%).^{32,34} There are no conclusive data showing that immunoglobulin changes the course of granulomatous complications of CVID.^{15,34}

Panel: Laboratory investigations for CVID in the patients who are HIV-negative in whom other causes of recurrent infections have been ruled out

Phase 1

- Complete blood count with differential
- Serum immunoglobulins—IgG, IgA, and IgM
- Urine protein analysis (to rule out loss of immunoglobulins due to nephrotic syndrome)

Phase 2

- IgG subclasses (IgG1 to IgG4; useful for patients with IgA deficiency or history of recurrent sinopulmonary infections)⁴⁹
- Functional antibody tests
 - Protein: diphtheria toxoid, tetanus toxoid, *H influenzae* B, isohaemagglutinins
 - Polysaccharide antigen: *S pneumoniae*
- T-cell, B-cell, and natural-killer cell quantitation by flow cytometry
- Possibly test for lymphocyte proliferation for mitogens and antigens (tetanus toxoid and *Candida*)

Phase 3

- B-cell subsets by flow cytometry* (to determine if there is a reduction in class-switched memory B cells, and changes in other B-cell subsets that correlates with certain clinical presentations^{35,50})
- Class-switched memory B cells (CD27+ IgD- IgM-)
- Non-switched memory B cells (CD27+ IgD+ IgM+)
- IgM-memory B cells (CD27+ IgM+ IgD^{dim})
- Transitional B cells (CD38+++ IgM++)
- Plasmablasts (CD38+++ M-)
- Mature B cells (CD19+ CD21+)
- CD21^{lo} B cells (CD19+ CD21^{lo})

Optional testing*

- Protein expression for BAFF-R, $\uparrow$ TACI, $\uparrow$ and CD19 $\uparrow$ on B cells and ICOS $\uparrow$ on activated T cells by flow cytometry
- Mutation analysis by gene sequencing for *TNFRSF13B* $\uparrow$ and ICOS $\uparrow$
- Mutation analysis for *TNFRSF13C* and CD19 are presently available only in specific research laboratories

*Tests may not affect diagnosis or management decisions but will provide additional information on the underlying basis for the CVID presentation. $\uparrow$ Tests are clinically available in specialised reference laboratories.

Gastrointestinal diseases

Gastrointestinal complications are fairly common in CVID (figure 1)—up to 50% of patients with CVID have chronic diarrhoea with malabsorption.^{15,25} Other gastrointestinal diagnoses in patients with CVID include Crohn's disease, intestinal granulomatous disease, intestinal parasitic bacterial or viral infections, coeliac sprue, and intestinal lymphangiectasia.¹⁵ The incidence of selective IgA deficiency in patients with coeliac disease is about ten-times higher than in the general population.^{38,39}

Neoplasias

Results of three large clinical studies including 248, 220, and 176 patients suggest that patients with CVID have a high risk of neoplastic disease—both haematological and solid-tumour (breast, prostate, ovary, skin, and colon). In particular, the incidence of lymphoma is increased in patients with CVID.^{15,40,41} The most common malignancies were non-Hodgkin lymphoma^{15,40} and gastric cancers.⁴⁰ Therefore, accurate clinical and family histories of neoplasia along with consideration of surveillance for malignancy, especially lymphoma and gastric cancer in patients with CVID, are appropriate. The surveillance approach has to be applied judiciously without indiscriminate and frequent use of radiological diagnostic procedures because some patients with CVID might have increased radio-sensitivity.⁴²

Differential diagnosis

When first assessing patients with recurrent infections or with suspected CVID, several alternative diagnoses must be considered. Due to the heterogeneous clinical presentation of CVID, investigation of anatomical anomalies of the lungs and sinus and of asthma and allergic rhinitis must precede further investigation into immune function. Other causes of hypogammaglobulinaemia that need to be ruled out include protein-losing enteropathy, nephrotic syndrome, haematological malignancies (including chronic lymphocytic leukaemia, multiple myeloma, primary amyloidosis, and non-Hodgkin lymphoma), and specific therapeutic drugs. Corticosteroids, gold salts, penicillamine, anti-malarial drugs, sulfasalazine, fenclofenac, phenytoin, and carbamazepine among other drugs can cause hypogammaglobulinaemia.⁴³ In patients taking steroids, hypogammaglobulinaemia is rarely associated with functional antibody defects.^{43–45}

Several other humoral immunodeficiencies present with hypogammaglobulinaemia and recurrent sinopulmonary infections, although most clinically relevant deficiencies usually present in infancy or childhood. These include X-linked agammaglobulinaemia,⁴⁶ autosomal-recessive agammaglobulinaemia, X-linked lymphoproliferative syndrome, and hyper-IgM syndromes (table 1). Each of these immunodeficiencies can be eliminated as a diagnostic possibility by combining clinical assessment with additional laboratory testing. A study of 60 male patients with CVID indicates that defects in *SH2D1A*, the gene encoding SH2 domain-containing protein 1A (SH21A; also known as SLAM-associated protein), associated with X-linked lymphoproliferative syndrome are very rare in patients with CVID, and follow-up testing is needed only for those with other associated clinical features of SH21A deficiency.⁴⁷ The onset of the clinical phenotype of X-linked lymphoproliferative syndrome is associated with a history of infection with Epstein-Barr virus.

Similarly, the possibility of X-linked agammaglobulinaemia presenting as CVID should be considered only in patients who present with less than 1% of the normal number of B cells, because this disorder is rare in adulthood.⁴⁸ Autosomal-recessive agammaglobulinaemia and hyper-IgM deficiencies are genetically heterogeneous, with five or six known defects each. Confirmatory testing (table 1) will enable identification of patients with specific genetic defects associated with these diseases. Selective IgA deficiency and selective IgG subclass deficiencies are typically asymptomatic but should be investigated if there is clinical evidence of recurrent infections without other features of CVID.

Diagnosis of CVID

The well-accepted definition of CVID includes three key features: the presence of hypogammaglobulinaemia of two or more immunoglobulin isotypes (low IgG, IgA, or IgM), recurrent sinopulmonary infections, and impaired functional antibody responses.^{4,8,15} The criteria for impaired functional antibody responses include absent isohaemagglutinins, poor responses to protein (diphtheria, tetanus) or polysaccharide vaccines (*S pneumoniae*), or both. In addition to these, there can be other clinical findings including autoimmunity, granulomatous disease, and neoplasia.

After obtaining relevant personal and family history and careful clinical examination, a systematic laboratory assessment (panel)^{49,50} should be done.⁴ Clinical examination should include assessment of key target organs, such as pulmonary-function testing, ear nose and throat review, and CT scans for sinusitis or bronchiectasis. Testing for gastrointestinal complaints and haematological anomalies is also useful. For patients presenting with recurrent bacterial sinopulmonary infections, in whom other causes of infections have been ruled out, one useful step-wise approach (panel) to the laboratory assessment of such patients would include (phase 1) a complete blood count, urine protein analysis, and measurement of serum immunoglobulin (IgG, IgA, and IgM) concentrations. The IgG concentrations in CVID are at least two SD below the mean for the patients' age^{8,18} and, in most cases, accompanied by low concentrations or absence of IgA,¹⁸ IgM, or both.

If there is hypogammaglobulinaemia, the next laboratory tests (phase 2) would include quantitative flow cytometric analysis of T, B, and natural killer cells, functional antibody responses to protein antigens (diphtheria toxoid, tetanus toxoid, *Haemophilus influenzae* –Hib, isohaemagglutinins) and polysaccharide antigens (*S pneumoniae* vaccine; panel). The importance of assessing functional antibody responses in these patients cannot be overstated because the results of these tests can determine whether patients require immunoglobulin replacement or not.⁵¹ If

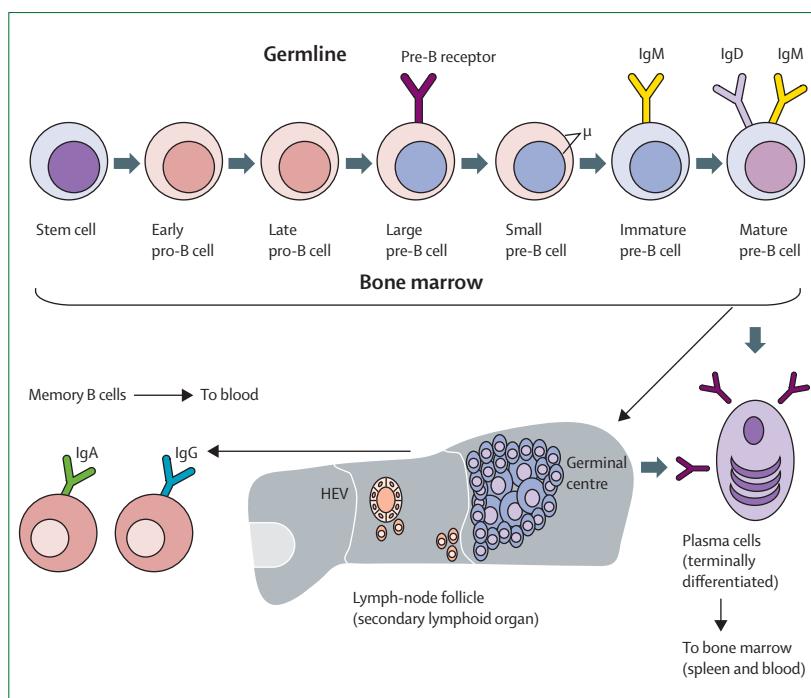


Figure 2: B-cell development and differentiation

B cells develop in bone marrow from pluripotent haemopoietic stem cells through rearrangement of immunoglobulin heavy-chain and light-chain genes and initial selection of the repertoire with selection against autoreactive B cells. Mature B cells expressing both IgM and IgD are exported from bone marrow and enter secondary lymphoid organs. Affinity maturation takes place through somatic hypermutation of the variable region genes in the germinal centre of the secondary lymphoid follicle where isotype class switching also takes place through class switch recombination, which enables the production of IgG, IgA, and IgE isotypes. B cells selected through affinity maturation can become either memory B cells or long-lived plasma cells that home back to the bone marrow and produce high-affinity antibodies. HEV=high endothelial venule.

patients with recurrent infections have normal concentrations of IgG and IgA, or IgA deficiency alone, then IgG subclass concentrations can be measured to determine if there is an IgG subclass deficiency.

Lymphocyte proliferative responses to mitogens and specific antigens, such as *Candida albicans* and tetanus toxoid, can also be measured, although this test is not essential for the diagnosis, because only 20% of patients with CVID have impaired proliferative responses,^{52–54} and these are often associated with reduction in the CD4 count associated with a normal to increased CD8 count, which can alter the ratio of CD4 to CD8.^{55,56} Numbers of natural-killer cells, as determined by flow cytometry, might also be low.⁵⁷

The cellular characteristics of the immune system in CVID are complex with several numerical and functional defects involving B cells, T cells, natural killer cells and macrophages and monocytes.^{57–62} The number of B cells in peripheral blood can be normal or reduced.^{15,63} T-cell abnormalities are common including decreases in number and function,^{15,64} defects in cytokine production,^{65,66} decreased T-helper-cell function,⁶⁷ abnormalities in T-cell signalling,^{68,69} diminished expression of the costimulatory molecule CD40 ligand,⁷⁰ and increased suppressor T-cell function.^{70,71}

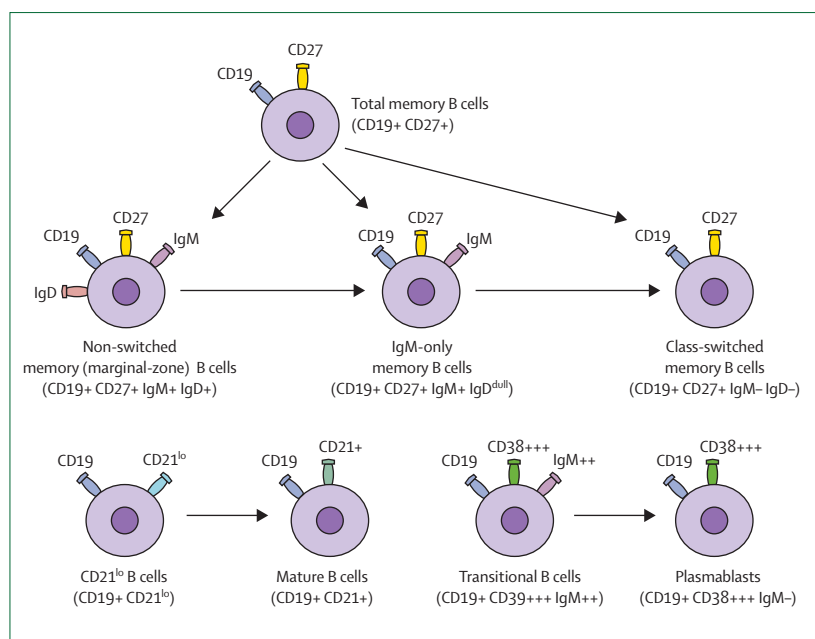


Figure 3: Peripheral blood B-cell subsets

The assessment of peripheral blood B-cell subsets is useful in the diagnosis of CVID. CD19 is a pan-B-cell marker that allows identification of all B-cell subsets in blood except normal plasma cells. CD27 typically indicates the memory phenotype and presence or absence of IgM and IgD differentiates memory subsets. The expression of CD38 and IgM distinguishes transitional B-cells and plasmablasts. CD21 is a marker for B-cell activation and is expressed on mature B cells. Multiparametric flow cytometry allows both absolute quantification and proportion analysis of these subsets.

If the clinical presentation and laboratory assessment (phase 1 and phase 2) are consistent with a CVID phenotype, then analysis (panel) for defects in the memory B-cell compartment and other peripheral B-cell subsets may provide further information (phase 3 testing; figures 2 and 3), particularly in relating changes in B-cell subsets, such as class-switched memory B cells, to clinical features of disease. The number of class-switched memory B cells (CD27+ IgM- IgD-)⁷² is low in 50–75% of patients with CVID;^{8,50,73,74} although it can also be low or the cells absent in other humoral immunodeficiencies, such as hyper-IgM syndrome.^{75,76}

In the past 5 years, the Paris⁷³ and Freiburg⁷⁴ classifications have attempted to define CVID with flow-cytometry techniques on the basis of the presence or absence of class-switched memory B cells. Very recently, however, data from the EUROclass trial³⁵ unified the two classifications and provided clinical links with results from the immunophenotyping of B-cell subsets (figure 3). The EUROclass data are from a multicentre European trial that assessed 303 patients with CVID and showed that severe reduction in the number of class-switched memory B cells is associated with granulomatous disease, splenomegaly, and autoimmune cytopenias.³⁵ Their results also showed that increases in other B-cell subsets, such as transitional B cells and CD21^{lo} B cells, were associated with lymphadenopathy and splenomegaly, respectively.³⁵

The origin and function of the various memory B-cell subsets in the humoral response has been ardently debated.^{77–82} However, there are enough data to prove a role for these memory-B-cell subsets in generating antibodies to both T-dependent and T-independent antigens,⁸³ and that changes in the B-cell memory compartment, due to an underlying immunodeficiency, could have substantial effects on the quality and quantity of the humoral immune response.

In CVID, the subgroups of B-cell defects that can be classified by flow cytometric analysis (panel) allow categorisation of patients on the basis of the underlying immune defect, although not all defects are clearly known. Different clinical laboratories have different approaches to diagnostic testing for CVID and clinical associations might therefore differ with the patients studied.

Classifications that take class-switched memory and non-switched memory B cells into account are useful for diagnosis.^{50,84} In one study, a reduction in the proportion of class-switched memory B cells was associated with a higher incidence of bronchiectasis, splenomegaly, and autoimmunity and was clinically more informative than either serum immunoglobulin concentrations or arbitrary grouping of patients as having CVID or specific antibody deficiency.⁵⁰ In another study, the absence or presence of IgM memory B cells (CD27+ IgM+ IgD^{dull}) and anti-IgM pneumococcal polysaccharide antibody responses subclassified patients with CVID into those with recurrent bacterial pneumonia and bronchiectasis and those without pneumonia and lung lesions, respectively.⁸⁴ Flow cytometric laboratory assessment of peripheral B-cell subsets can be made more amenable to routine clinical diagnostic testing by use of whole blood instead of isolated peripheral-blood mononuclear cells.⁸⁵

Genetic defects in CVID

In the past 5 years, investigators have described defects in four genes associated with CVID—inducible T-cell costimulator (*ICOS*),^{86,87} tumour necrosis factor receptor superfamily, member 13B (*TNFRSF13B*, also known as *TACI*),^{88–91} tumour necrosis factor receptor superfamily, member 13C (*TNFRSF13C*, also known as *BAFFR*),⁹² and *CD19*.⁹³ (table 2).^{94–96}

ICOS

The first reported genetic defect associated with CVID and characterised by a deficiency of ICOS, which is expressed on activated T cells, was identified in nine individuals with CVID from four apparently unrelated families.^{86,87} These nine patients with mutations in *ICOS* presented with recurrent bacterial infections, splenomegaly, autoimmune neutropenia, intestinal lymphoid hyperplasia, and neoplasia.⁹⁶ ICOS-deficient patients have few peripheral B cells, few or no class-switched memory B cells, and hypogammaglobulin-

	Inheritance	B-cell-subset analysis by flow cytometry	Protein expression on cell surface
<i>TNFRSF13C</i> (<1% of CVID cases) ^{92,94}	Autosomal recessive	Reduced class-switched and non-switched memory B cells with increased transitional B cells	BAFF-R expression is absent on B-cell surface
<i>TNFRSF13B</i> (10–20% of CVID cases) ^{88–91,95*}	Autosomal dominant	Low to absent IgA, autoimmune disease, lymphoproliferative disease, splenomegaly, reduced class-switched memory B cells	95% have normal TACI expression on B-cell surface, <5% have absent TACI expression
<i>ICOS</i> (~2%) ^{86,87,94,96}	Autosomal recessive	Reduced class-switched memory B cells, nodular lymphoid hyperplasia, autoimmunity, predisposition to neoplastic disease	ICOS expression on the surface of activated T cells is absent
<i>CD19</i> (<1%) ⁹³	Autosomal recessive	Decrease in class-switched memory B cells, low CD21 expression on B cells, normal numbers of CD20+ mature B cells in peripheral blood	Low to absent expression of CD19 protein on the surface of CD20+ B cells

BAFFR=B-cell-activating-factor-family receptor. *And our unpublished data.

Table 2: Gene defects in CVID, inheritance, and associated laboratory phenotype

aemia.⁸⁷ T cells from ICOS-deficient patients produce very little interleukin 10, which may be associated with the defective formation of germinal centres leading to impaired B-cell memory (figure 4).⁹⁴

The defect seems to be inherited as an autosomal-recessive trait because all nine patients have the same homozygous deletion in *ICOS*. Heterozygous parents and siblings of patients deficient in *ICOS* are asymptomatic, and genetic analysis of these nine patients indicates a common founder ancestor,⁸⁷ suggesting that *ICOS* mutations cause CVID in only a few patients. About 2% of patients with CVID have defects in this gene.⁹⁴

ICOS is upregulated on both CD4 and CD8 effector and memory T cells⁹⁷ and activated natural-killer cells, and it enhances natural-killer-cell function. The stimulator's ligand is expressed on lymphoid and non-lymphoid tissue, whereas ICOS is expressed constitutively in germinal centres and T-cell zones of spleen, lymph nodes, and Peyer's patches.⁹⁸

Tumour necrosis factor superfamily

Two groups independently identified mutations in *TNFRSF13B* in 17 patients with CVID and one with sIgAD in 2005.^{89,91} Mutations in TACI, the protein encoded by *TNFRSF13B*, are associated with a clinical phenotype of lymphoproliferation, which may include splenomegaly or tonsillar hyperplasia, and IgA deficiency, with autoimmune thyroiditis being reported in 15% of patients with CVID.^{89,91}

The clinical relevance of these mutations was recently further analysed.^{88,90} Pan-Hammarstrom and colleagues⁹⁰ studied 424 patients with CVID and sIgAD and 2209 healthy people from Sweden, Germany, and the USA. The differences between patients and healthy people in prevalence of some mutations in TACI, the protein encoded by *TNFRSF13B*, such as the Cys104Arg, Ala181Glu and 204insAla (also known as Leu69fsX11) was statistically significant, indicating that these mutations even in heterozygous states are associated with CVID; but they do not seem to be associated with

sIgAD. An Arg202His mutation, however, was significantly associated with sIgAD. Prevalence of several other mutations did not differ significantly between patients and controls.⁸⁹

In a second study of mutation in TACI, 212 patients with CVID were compared with 124 healthy controls. Only Cys104Arg and Ala181Glu mutations were significantly associated with CVID.⁸⁸ The Arg181Glu mutation is present in some healthy people but is significantly rarer than in patients.⁸⁸ These healthy controls might develop CVID later in life.¹⁵

In families with dominant inheritance of the Ala181Glu mutation, some people with the mutation are completely asymptomatic, suggesting that the mutation has incomplete penetrance.^{90,91} In a very recent study of 176 patients with CVID by Zhang and co-workers,⁹⁵ 13 patients had heterozygous mutations in TACI. Five of these 13 patients had the Cys104Arg mutation, while another two were compound heterozygotes with the Cys104Arg and Ser144X or Ser194X mutations. Three of the 13 patients had the Ala181Glu mutation and a fourth was a compound heterozygote for Ala181Glu and Leu171Arg mutations.⁹⁵ The remaining two patients had a Cys172Tyr and Arg72His mutation respectively. A previous study did not find significant association for either Arg72His or Leu171Arg, which were found in two and one of 212 patients, respectively, but not in any of 124 healthy people.⁸⁸

In a smaller group of 53 patients with CVID assessed at the Mayo Clinic (unpublished), we found six patients with heterozygous TACI mutations: five had the Ala181Glu and one the Cys104Arg substitutions. Of these patients, two were brothers, indicating a familial inheritance. From these various studies, about 10–20% of patients with CVID seem to have mutations in *TNFRSF13B*.

Zhang and co-workers⁹⁵ recently showed that the presence of a heterozygous mutation in *TNFRSF13B* alone does not seem to cause CVID. Whether this finding is due to incomplete penetrance or delayed onset, or whether additional genetic–environmental factors are

required for the manifestation of an immune phenotype, is not entirely clear.

Mutation of TNFRSF13C, which encodes BAFF-R, has been described in only one individual with CVID aged

60 years who had a homozygous 24 base-pair deletion.⁹² In this patient, the mutation was associated with distinct anomalies in peripheral B-cell subsets with profound reduction of both class-switched (CD27+ M- D-) and

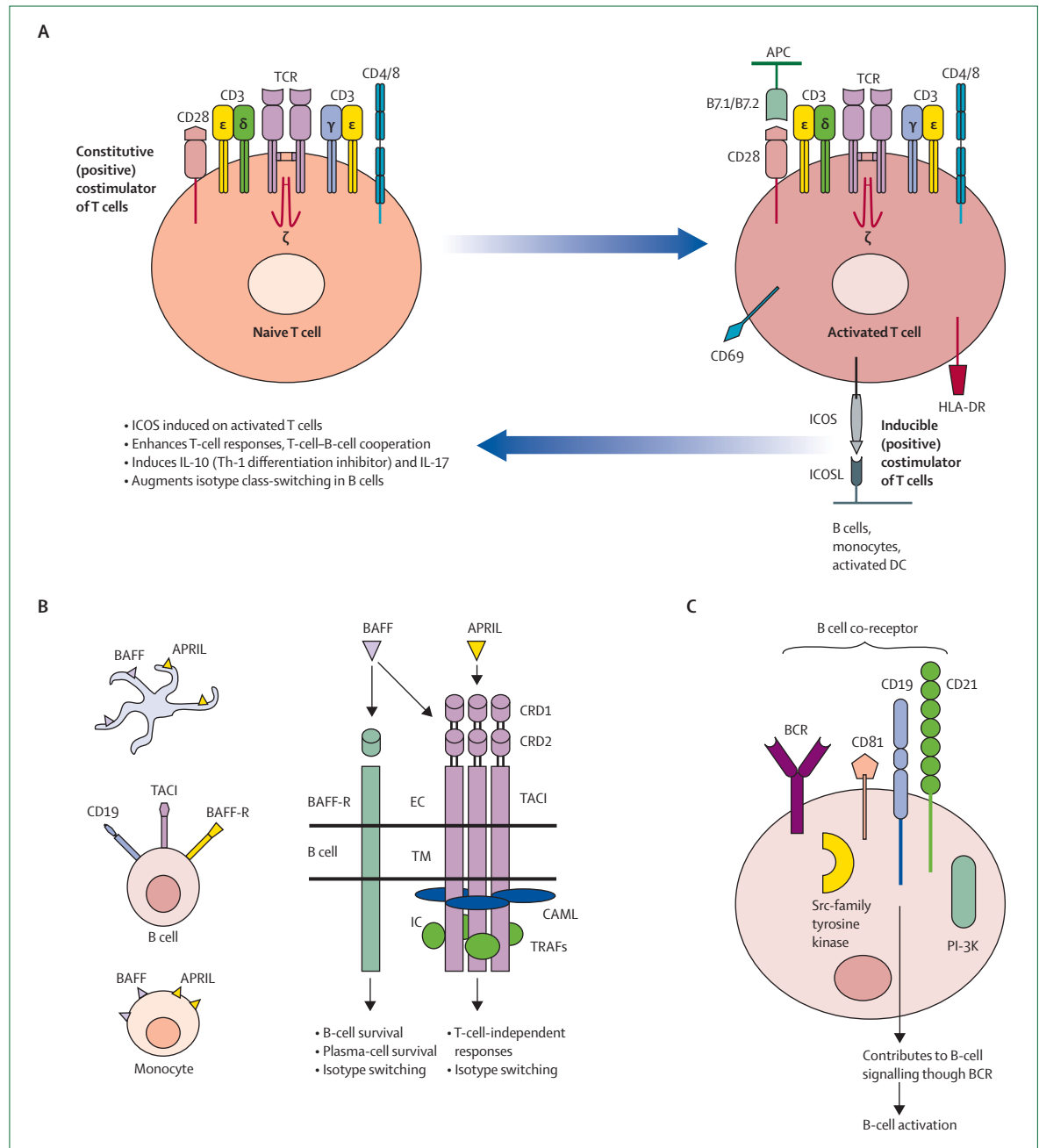


Figure 4: Molecules implicated in genetic studies of CVID

(A) ICOS is a positive costimulator (like the constitutively expressed CD28) that enables T-cell interactions with B cells, monocytes, and dendritic cells. (B) BAFF-R and TACI are cell-surface receptors belonging to the TNF-receptor family that play a part in B-cell differentiation and function. BAFF-BAFF-R interactions provide critical survival signals for differentiation of peripheral B cells. The role of BAFF-TACI interactions is less clearly defined. TACI signals intracellularly through the TNF receptor-associated factors (TRAF) to induce nuclear factor- κ B activation.¹⁰¹ TACI also interacts intracellularly with calcium modulator and cyclophilin ligand (CAML). Through interaction with APRIL, TACI regulates isotype class switching of immunoglobulins and the antibody response to T-independent antigens.^{89,94,99,100} (C) CD19 is a B-cell-specific cell-surface marker that is part of the B-cell coreceptor along with CD21 and CD81;⁹³ CD19 is expressed throughout B-cell maturation from pro-B cells through to plasmablasts, before CD21 or CD81, it is not expressed on plasma cells. Coligation of the B-cell receptor (BCR) with the coreceptor complex of CD19-CD21-CD81 increases B-cell signalling by several thousand times.¹⁰²

non-switched memory or marginal-zone (CD27+ M+ D+) B cells with an increase in the transitional B-cell compartment (CD38+++ M++) and a decrease in plasmablasts (CD38+++ M-). Preliminary evidence of other patients with this mutation from clinical presentation and flow cytometric laboratory analysis needs to be investigated with genetic testing.

TACI and BAFF-R ligands are expressed on macrophages, monocytes, and dendritic cells.^{99,100} BAFF-R interactions provide crucial survival signals for differentiation of peripheral B cells whereas TACI induces nuclear factor κ B activation¹⁰¹ and regulates class-switching of immunoglobulins and the antibody response to T-independent antigens (figure 4).^{89,94,99–101}

CD19

Four patients with CVID from two unrelated families had homozygous mutations in *CD19*, resulting in undetectable CD19 protein expression on B cells in one patient and reductions in the level of expression in the other three.⁹³ Three of the four patients were siblings and were diagnosed as adults, although they had been symptomatic during childhood. The fourth patient was diagnosed at age 10 years after recurrent infections starting in infancy. In patients with this defect, the total number of B cells (CD20+) in blood is normal with low or undetectable surface expression of CD19, and the numbers of CD27+ memory B cells and CD5+ B cells are decreased (figure 4).^{93,102} *CD19* knockout mice have deficient antibody responses to most antigens. CD19 is expressed on B cells from an early stage of development and therefore seems to play a part in signalling through the B-cell receptor even without forming a complex with CD21 and CD81.

Genetic testing

The genetic defects we have described account for only a few patients with CVID (table 2), and nearly 75% of patients have no known defect.

Flow cytometry screening for protein expression for the four proteins implicated is available in specialised laboratories in the USA and Europe. However, in the case of mutations in *TNFRSF13B*, less than 5% of patients have abnormal levels of protein expression on the cell surface. The remaining *TNFRSF13B* mutations are associated with functional defects. Therefore, flow cytometry for *TNFRSF13B* expression is likely to be uninformative in most cases.

Although genetic testing is at the forefront of diagnosis for primary immunodeficiencies, its use in CVID has to be cautious and clinically justified due to both the expense and the medicolegal ramifications for both patients and family members. At present, the diagnosis and treatment of CVID do not require specific knowledge of the underlying genetic defect; however, determination of the genetic defect helps to understand the biology and epidemiology of the disease. Other reasons for

genetic testing would be to enable early management of complications associated with specific genetic defects and to develop robust genotype–phenotype correlations in a specific population of patients, which have not always been reproducible in genetic studies.¹⁰³ Also, genetic testing may be helpful in the investigation of familial cases of CVID, since two of the four genetic associations (*ICOS* and *CD19*) reported so far have a strong family-based association. In the case of *TNFRSF13B* mutations, only a few patients have evidence of familial bias, and most family members with mutations are asymptomatic.⁹⁵

Treatment and clinical management

Treatment of CVID

The main goal of therapeutic management in CVID is to decrease the morbidity and mortality associated with recurrent infections. Intravenous immunoglobulin is effective^{104–106} and is currently the mainstay of therapy for CVID.⁸ Intravenous immunoglobulin also reduces the incidences of pneumonia¹⁰⁷ and serious recurrent bacterial infections^{15,107} and prevents chronic lung disease and enteroviral meningoencephalitis.¹⁰⁸

Immunoglobulin replacement can be given either subcutaneously or intravenously.¹⁰⁹ The current dosing recommendations for intravenous immunoglobulin are 300–400 mg/kg body weight, every 3–4 weeks⁸ with the IgG concentration maintained above 5 g/L;¹¹⁰ although some patients may benefit from a higher trough concentration, closer to 7 g/L. Alternatively, subcutaneous delivery of immunoglobulin with slightly more than a quarter of the monthly dose each week is therapeutically comparable with intravenous dosing.^{111,112}

There can be both mild and serious adverse reactions to the use of intravenous immunoglobulin. The minor adverse reactions to intravenous immunoglobulin include headache, nausea, malaise, myalgias, arthralgias, chills, anxiety, flushing, abdominal cramps, rash, low-grade fever, and leukopenia. Most of these can be prevented by slowing of intravenous immunoglobulin infusion, premedication of patients with 500–1000 mg of paracetamol and 25–50 mg of diphenhydramine orally, or both. The rarer but more serious side-effects of intravenous immunoglobulin include anaphylaxis,¹¹³ acute renal failure,¹¹⁴ stroke,¹¹⁵ myocardial infarction,¹¹⁶ deep venous thrombosis or pulmonary embolus,^{117,118} and aseptic meningitis.¹¹⁹

Patients with CVID who have IgA deficiency (IgA <0.07 g/L) typically receive IgA-deficient blood products. The need for exclusive use of IgA-deficient preparations has been controversial.^{120–122} However, patients who have anti-IgA antibodies²⁴ and meet clinical criteria for replacement therapy should also be considered for IgA-depleted immunoglobulin products because of a potential increased risk of anaphylactic reactions.^{11,121,122} The use of subcutaneous immunoglobulin replacement

might lower the risk of many of the side-effects associated with intravenous immunoglobulin. The minor reactions of subcutaneous immunoglobulin replacement are local inflammation at the infusion site and, rarely, fever, chills, and cold sweats.^{112,123}

Antimicrobial drugs also play an integral part in the treatment of CVID,⁸ because intravenous immunoglobulin alone is not enough to prevent or eradicate all active infections.¹²⁴ The use of fluoroquinolones and amoxicillin clavulanate are effective in managing the sinopulmonary infections in CVID. The role of antimicrobial prophylaxis in addition to intravenous immunoglobulin has not been definitively established and needs further study.¹²⁵

Autoimmunity and neoplasia associated with CVID are commonly treated as per standard clinical practice in patients without CVID,⁸ although theoretical risks of additional immunosuppression exist. In particular, the treatment of autoimmune and granulomatous diseases in CVID presents a profound therapeutic challenge. Corticosteroids are effective in combating many of the autoimmune and granulomatous manifestations, but the side-effects of corticosteroids may limit its long-term efficacy. New-generation monoclonal antibodies have been used to treat some of the autoimmune and granulomatous complications in CVID, but no systematic double-blind, randomised clinical trials have investigated their efficacy and safety. Several case reports describe the clinical usefulness of monoclonal antibodies. For example, infliximab has been used for Crohn's disease associated with CVID¹²⁶ and also for caseating granulomatous disease.^{127,128} Rituximab has been used with some success in medically refractory severe autoimmune thrombolytic purpura¹²⁹ and autoimmune haemolytic anaemia.¹³⁰ Etanercept has been used to treat scarring alopecia caused by sarcoidal granulomas in patients with coexisting juvenile rheumatoid arthritis and CVID.¹³¹

Vaccinations, surveillance, and education

Because CVID is most commonly diagnosed in adulthood, many patients are likely to have previously received live vaccines for infectious diseases. Additional immunity is provided for patients treated with intravenous or subcutaneous immunoglobulin because circulating antibodies are present in these preparations. However, the measles-mumps-rubella and varicella vaccines are not recommended in patients receiving replacement immunoglobulin therapy, because the vaccines may be inactivated by the presence of neutralising antibodies.^{8,132} Inactivated vaccines can be given to patients with CVID but these may not be effective because of the underlying antibody deficiency.⁸ Because influenza is unlikely to be represented in the replacement-immunoglobulin, the inactivated-subunit influenza vaccine is commonly recommended yearly as a prophylactic.

Lifetime surveillance for cancer and autoimmunity after the diagnosis of CVID is important so that effective intervention can be started early if necessary. Surveillance should include physical assessments and blood tests when appropriate. Endoscopy may also be useful in this screening protocol for the detection of gastric carcinoma or mucosa-associated lymphoid-tissue lymphoma. Patients should be assessed by physicians familiar with their immunodeficiency history every 6–12 months.⁸ As with all chronic diseases, history and the physical examination should guide appropriate investigations. For those patients who remain healthy on replacement immunoglobulin, we recommend, at a minimum, age-appropriate cancer screening as endorsed for the general healthy population, such as colonoscopy, prostate examination, pap smears, and mammograms. There are currently no formal recommendations on the frequency of radiography for cancer surveillance.

Finally, education on and raising the awareness of the medical and social implications CVID are crucial for both patients and family members, for whom the rarity and substantial complexity of this disease can pose a significant emotional and financial burden. There are several resources available in the USA and Europe that provide educational and social support for patients with primary immunodeficiencies and their families; these include the Immune Deficiency Foundation and the Jeffrey Modell Foundation.

Summary and recommendations

Although the 2005 practice guideline for the diagnosis and management of primary immunodeficiency was developed for allergy and immunology specialists and not family doctors, it offers helpful information on the clinical and laboratory assessment of patients with several kinds of immunodeficiency disorders, including humoral immunodeficiencies. Algorithm 2 in the 2005 practice guidelines⁸ offers a global diagnostic algorithm for the assessment of antibody-related primary immunodeficiencies, although it does not provide specific information on the clinical usefulness of new laboratory tests, such as flow-cytometric B-cell subset analysis and genetic testing in the assessment of CVID.

In summary, clinicians should consider CVID as a possible diagnosis when assessing patients with frequent bacterial sinopulmonary infections. There should be judicious use of laboratory tests when assessing such patients to prevent unnecessary testing and expense. Many of the new and advanced laboratory tests, such as peripheral-blood B-cell-subset studies, specific protein analysis, and genetic testing for CVID-associated mutations, are now clinically available in specialised centres to aid in the diagnosis and management of CVID. Because most patients with primary immunodeficiencies are first seen by family doctors, the goal of these recommendations for clinical

For the Immune Deficiency Foundation see <http://www.primaryimmune.org>

For the Jeffrey Modell Foundation see <http://www.jmfworld.org>

For the 2005 practice guidelines see http://www.jcaai.org/pp/pp-immunodeficiency-update_05-0602.pdf

and laboratory assessment of patients with CVID is to promote prompt and accurate diagnosis in this setting.

Contributors

MAP and RSA contributed equally to the preparation and writing of this Seminar. MAP participated in the planning, writing, and editing of the paper and approved the submitted version. JTL, JBH, and DEM participated in the conception, reviewing and editing of the paper and approved the submitted version. RSA was involved in the conception and preparation of the paper at all stages, including the reference search, writing the text, preparation of figures, and editing.

Conflict of interest statement

MAP and JBH are coinvestigators in a multicentre extension study on the safety and efficacy of IgPro10 in patients with primary immune deficiency sponsored by ZLB Behring; MAP, JBH, and DEM are coinvestigators in a phase III open-label, prospective, multicentre study of the efficacy, tolerability, safety, and pharmacokinetics of immune globulin subcutaneous IgPro20 in patients with primary immunodeficiency sponsored by ZLB Behring; MAP, JTL and JBH were coinvestigators in ZLB03_002CR, a multicentre study of the efficacy, safety, and pharmacokinetics of IgPro10 in patients with primary immunodeficiency, sponsored by ZLB Behring—none of the investigators received personal funding for their involvement in the above studies. RSA received a USIDNET (US Immunodeficiency Network funded by the NIH) and Jeffrey Modell Foundation travel scholarship to attend the IUIS/WHO meeting on primary immunodeficiencies in 2007.

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