Clinical aspects of Common Variable Immunodeficiency

There is more to the image than meets the eye

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Klinische aspecten van Common Variable Immunodeficiency

Er is meer dan je met het blote oog kan zien

(met een samenvatting in het Nederlands)

PROEFSCHRIFT

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Liselotte Jacobien Maarschalk- Ellerbroek
geboren op 1 mei 1970 te Amsterdam

Promotor: Prof. dr. A. I.M. Hoepelman

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Contents

1

Introduction

The primary antibody deficiency syndromes are a heterogeneous group of inherited or acquired disorders of different etiologies and represent the largest group (50-70%) (1) of primary immunodeficiencies across all age groups (2). The best known primary antibody deficiencies are IgA deficiency, common variable immunodeficiency (CVID), X-linked agammaglobulinaemia (XLA), immunoglobulin G (IgG) subclass deficiency, and selective antibody deficiency with normal immunoglobulins (SADNI). Primary antibody deficiencies are characterized by B-cell dysfunction and share the feature of recurrent respiratory tract infections with encapsulated bacteria such as *Streptococcus pneumoniae* and *Haemophilus influenzae* but other infections are also associated with the particular syndromes.
In this thesis we will address the clinical and immunological aspects mainly of CVID which is the most complicated primary antibody deficiency and we will compare them to patients with other primary immunodeficiencies such as X-linked agammaglobulinaemia (XLA), immunoglobulin G (IgG) subclass deficiency, and selective antibody deficiency with normal immunoglobulins (SADNI).

Common variable immunodeficiency (CVID)

Common variable immunodeficiency (CVID) is the most heterogeneous disease among the primary antibody deficiency syndromes and has a prevalence of 1:10.000 to 1:100.000 (3-5). The current European Society for immunodeficiencies (ESID)/ Pan-American Group for Immunodeficiency (PAGID) diagnostic criteria (6;7) state that CVID is probable in a patient with recurrent infections, a marked decrease of immunoglobulin (Ig) G (at least 2 SD below the mean for age) and a decrease in at least one of the isotypes IgM or IgA, and fulfills all of the following criteria:

1. Onset of immunodeficiency at greater than 2 years of age,
2. Absent isohemagglutinins and/or poor response to vaccines,
3. Defined causes of hypogammaglobulinaemia have been excluded.

In most CVID patients the symptoms will start between the second and fourth decade of life (8), however, the diagnosis is often made when

the patient has already reached adulthood. A diagnostic delay is very common with a mean of 6–8 years after the onset of symptoms (8-11), but it can take as long as a decade before the appropriate diagnosis is made (10). The diagnostic and subsequent delay in (immunoglobulin) therapy is thought to be a major cause of the development of organ damage resulting in increased morbidity and mortality (10-13;13;14).

Multiple immunological abnormalities have been described in CVID patients (15-18). The principal defect is a failure in B cell differentiation leading to reduced serum immunoglobulin (Ig) levels and an abnormal antibody response to vaccination(19). In addition T cell dysregulation at various levels may also be present (8;20-30). Although the immunological abnormalities may be related to the pathogenesis of the disease, it is also possible that they represent epiphenomena as a result of the disease process. Approximately 10 % of cases of CVID demonstrate familial clustering (31), however, in the majority of patients a genetic defect has not been established. The rate of progress in unraveling the genetic basis of CVID has progressed, mutations in inducible co-stimulator (ICOS) gene have been identified as genetic disorder resulting in the CVID phenotype (32). Furthermore, mutations have been detected in various B cell related the tumor necrosis factor family members (TNFRSF) member genes (TACI and BAFF-R) (33-35), in members of the CD19-B cell receptor complex (CD19, CD21 and CD81) (36-38) and in the B cell differentiation antigen, CD20 (39). In addition, polymorphisms in genes involved in DNA metabolism (MSH5, MSH2, MLH1, RAD50, and NBS1) (40;41) have also been identified in CVID cohorts. Some of these genetic mutations are likely to be disease causing (ICOS, CD19, CD20, CD81) whereas the others (TACI, BAFF receptor, Msh5) are likely to require additional genetic contributions as the genetic mutation alone does not necessarily lead to a CVID phenotype.

The typical presentation of CVID patients is that of recurrent bacterial upper and lower respiratory tract infections (8;9) with *Streptococcus pneumoniae* and *Haemophilus influenza* as the most common (encapsulated) organisms isolated (8;13). Furthermore, gastrointestinal infections caused by *Giardia Lamblia*, *Salmonella*, and *Campylobacter* and

more unusual infections with mycoplasma species (42), enteroviruses (43;44) and ureaplasma may occur (45). The mainstay of therapy is immunoglobulin replacement therapy, possibly in combinations with antibiotics prophylaxis. Replacement therapy with immunoglobulins increases life expectancy and reduces the frequency and severity of infections, but the effect on end-organ damage is still unknown.
The largest problem for CVID patients is the development of non-infectious complications that are associated with excess morbidity and mortality (14) such as end-organ damage due to recurrent infections. Other non-infectious complications include lymphoproliferative (granulomatous) disorders (46), autoimmune disease (47) (typically auto-immune cytopenia, but also rheumatoid arthritis, and organ-specific autoimmunity), and an increased risk of cancer, especially malignant lymphoma (48-50). These non-infectious complications are probably due to the underlying immune dysregulation (8;10;51). The pulmonary and gastrointestinal tract are most frequently affected by these complications and have been the main focus of research of this thesis. Chronic pulmonary diseases may result from recurrent infections and/or immune dysregulation. Most common are structural airway disease and interstitial lung disease (ILD) (52). Structural airway disease is generally the result of recurrent and lower respiratory tract infections causing bronchiectasis. Bronchiectasis has been described in 4–76% of CVID patients (8-10;45;53-55) and are associated with recurrent infections, a delayed diagnosis of CVID and inadequate treatment (53;54). However, it has been shown that structural airway disease may still develop or progress despite the absence of infections and adequate immunoglobulin replacement therapy (9;53). ILD includes granulomatous lung disease, lymphoid interstitial pneumonia, organizing pneumonia and lymphoproliverative disorders and is associated with immune dysregulation, mainly T-cell dysfunction and autoimmune disease (46;56;57). Treatment consists of steroids or in rare cases with other immunomodulatory medications (58;59). Furthermore, in CVID patients the gastrointestinal tract is also frequently affected and can involve the entire gastrointestinal tract (60;61). Diarrhoea is common and occurs periodically in about

20-60% of patients (8;60;62-65). The spectrum of non-infectious GI disease in CVID includes autoimmunity (pernicious anaemia), chronic inflammatory conditions (atrophic gastritis, villous atrophy, colitis) and nodular lymphoid hyperplasia (60;64;66). Finally, a relative high risk of gastrointestinal malignancy has been observed in CVID patients, especially gastric cancer (48;50).
While 32-50% of the CVID patients remain relatively free of non-infectious conditions (10;14), a subgroup will develop one or more of these CVID related complications. Attempts have been made to classify the heterogeneous CVID population into subgroups based on clinical parameters (10;67) and flow cytrometric markers of B-(51;68;69) and T cells (70;71). This classification may help to define variables that might predict which particular patients will be prone to complications and to improve clinical follow up and treatment. Clinically, patients have been divided in the following distinct phenotypes: 1. no complications (infections only), 2. autoimmunity, 3. polyclonal lymphocytic infiltration, 4. enteropathy and 5. (lymphoid) malignancy (10;67). Different B cell classification schemes have been proposed (68;69;72) in which a severe reduction in switched memory B cells was associated with a higher risk of granulomatous disease and splenomegaly (51). Transitional B cell elevation was associated with a greater risk of lymphadenopathy. Furthermore, a reduction of CD4 naive T cells (73-76) was associated with autoimmunity and lymphoproliferation (73;75), splenomegaly and granulomatous disease (76).
The genetic and clinical characteristics of CVID are currently under active investigation.

IgG subclass deficiency and Selective antibody deficiency with normal immunoglobulins (SADNI)

IgG subclass deficiency and Selective antibody deficiency with normal immunoglobulins (SADNI) are clinically less severe and much more common primary antibody deficiencies.
A clinically significant IgG subclass deficiency is defined as recurrent sinopulmonary infections, reduced levels of one or more IgG subclasses (IgG1–4) and an inadequate response to vaccination (6). About half of

all patients with a clinically significant IgG subclass deficiency have a IgG2 deficiency (77). In 20% of cases however, the finding of lower IgG subclass levels is merely a laboratory finding that does not necessarily lead to symptoms (78).
SADNI is classified as recurrent sinopulmonary infections with an abnormal response to polysaccharide vaccination in the presence of normal antibody levels (79;80). The prevalence of SADNI is 5–10% in children over 4 years of age with recurrent infections (81;82) and 8% in adult patients with recurrent pneumonia (83). Non-infectious complications have not been described in patients with IgG subclass deficiency and SADNI. Nevertheless, these conditions occasionally progress to CVID (84;85).

Congenital agammaglobulinaemia

X-linked agammaglobulinaemia (XLA) is rare but the most frequent type of congenital agammaglobulinaemia. XLA is a hereditary primary antibody deficiency first described in 1952 (86) and is caused by mutations in the gene for Bruton tyrosine kinase (*BTK)* that is vital for B-cell development (87;88). A mutation in the BTK gene will result in the deficient development of B lymphocytes and subsequent deficiency of all Ig isotypes (87;89). XLA occurs in a frequency of about 1 in 100,000 in the male population. Before the era of immunoglobulin replacement therapy only few if any XLA patients survived past infancy or early childhood. An early diagnosis, immunoglobulin therapy, and the prompt use of antibiotics have improved the prognosis dramatically in the last decades (90). XLA patients usually present with recurrent bacterial infections at a very young age which is often complicated by the development of pulmonary damage (bronchiectasis, fibrosis). Other infections can be chronic and unremitting systemic infections with enteroviruses (43;91), mycoplasma spp. and ureaplasma spp. as well as chronic gastroenteritis caused by rotaviruses or *Giardia lamblia* (87;89;92). Furthermore, a variety of malignancies have been reported, including lymphoreticular malignancies (89;93) and gastric and colorectal carcinoma (94-97). Non-infectious complications, such as autoimmunity or auto inflammatory disease are uncommon (98).

Scope and aims of this thesis

The aim of this thesis was to study the prevalence of the different CVID related complications in adult patients and to assess the relation between clinical parameters, therapeutic measures and B- and T cell parameters in order to predict which patients are prone to complications.
The pulmonary and gastro intestinal tract are most frequently affected by these complications and are associated with high morbidity and mortality and have therefore been the main focus of research of this thesis. Eventually, this may lead to a better recognition of risk factors for the development of CVID related complications and may improve diagnosis, follow-up and treatment of patients.

Outline of the thesis

I. The mainstay of therapy for patients with primary antibody deficiency is the use of prophylactic antibiotics and/or Ig replacement therapy. Although replacement therapy with immunoglobulins has shown to increase life expectancy and reduce the frequency and severity of infections, the effect on end-organ damage is still unknown. In *chapter 2* aspects of Ig replacement therapy in primary antibody-deficient patients will be addressed.

II. In *chapter 3* we determined the spectrum and prevalence of clinical manifestations and immunological characteristics during the long term follow-up of adult patients with an antibody deficiency in the outpatient clinic of our university hospital.

III. The gastrointestinal tract is frequently affected in patients with CVID; however, the exact frequency of gastrointestinal pathology and its related symptomatology is largely unknown. Likewise, no data are available on the role of endoscopic screening in asymptomatic patients. Given the assumed risk and implications of gastrointestinal malignancies in patients with CVID and XLA, routine evaluation of the gastrointestinal tract in all patients has been suggested by some

investigators but how often is not clear (98;99). Others advice screening only in case of symptoms (100). In *chapter 4* we aimed to determine (subclinical) gastrointestinal disease by endoscopic screening in a cohort of CVID and XLA patients, irrespective of the presence of gastrointestinal symptoms.

IV. Chronic pulmonary disease in CVID patients has been associated with an increased mortality (10) and therefore early detection and monitoring of progression will be essential to prevent progressive lung disease by additional therapeutic measures. In *chapter 5* we determined the prevalence of subclinical pulmonary disease in CVID patients by thin slice chest CT and a standardized scoring system and we will correlate these findings with the results of pulmonary function tests and IgG trough levels.

V. Chronic pulmonary and gastrointestinal disease has been associated with an excess morbidity and early mortality in the affected CVID patients (9;10;14). Early detection and monitoring of progression of such conditions is therefore essential. In *chapter 6*, peripheral blood B and T lymphocyte subsets were analysed in a cohort of CVID patients with well-defined gastrointestinal- and pulmonary pathology, in order to identify patients at risk for gastrointestinal- and pulmonary complications.

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Immunoglobulin treatment in primary antibody deficiency

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Abstract

The primary antibody deficiency syndromes are characterised by recurrent respiratory tract infections and the inability to produce effective immunoglobulin (Ig) responses. The best known primary antibody deficiencies are common variable immunodeficiency (CVID), X-linked agammaglobulinaemia (XLA), immunoglobulin G (IgG) subclass deficiency, and selective antibody deficiency with normal immunoglobulins (SADNI). Therapy in these patients consists of prophylactic antibiotics and/or Ig replacement therapy. Diagnostic delay remains common owing to limited awareness of the presenting features and may result in increased morbidity and mortality. Replacement therapy with immunoglobulins increases life expectancy and reduces the frequency and severity of infections, but the effect on end-organ damage is still unknown. Both intravenous immunoglobulin (IVIg) and subcutaneous immunoglobulin (SCIg) treatment appear to be safe, with comparable efficacy. A starting dose of 300–400 mg/kg/month in IVIg and 100 mg/week for SCIg is recommended. IgG trough levels should be >5 g/L for patients with agammaglobulinaemia and 3 g/L greater than the initial IgG level for patients with CVID; however, the clinical response should be foremost in choosing the dose and trough level. Infusion-related adverse reactions are generally mild owing to improved manufacturing processes. In this paper, aspects of Ig replacement therapy in primary antibody-deficient patients will be addressed.

Introduction

The primary antibody deficiency syndromes represent the largest group of primary immunodeficiencies. Multiple molecular defects have been identified in the pathways involved in B-cell development; in a US study, B-cell defects related immunodeficiencies comprised 78% of primary immune deficiencies (1). Primary antibody deficiencies share the feature of recurrent upper and lower respiratory tract infections (RTIs) with encapsulated bacteria such as *Streptococcus pneumoniae* and *Haemophilus influenzae*, but other infections may also be associated with the particular syndromes.

Common variable immunodeficiency (CVID) is the most common primary antibody deficiency. It is defined as the triad of recurrent respiratory (and/or gastrointestinal) infections, a reduction in immunoglobulin G (IgG) levels (total IgG >2 standard deviations below the mean for age), IgA and/or IgM levels, and a reduced antibody response to vaccination. CVID represents a heterogeneous disease spectrum that may also involve autoimmune phenomena, chronic granulomatous and inflammatory organ disease, and an increased risk of cancer. Diagnostic delay is very common, with a mean of 6–8 years after the onset of symptoms (2;3), but it can take as long as a decade before the appropriate diagnosis is made. The principal defect in CVID is a failure in B-cell differentiation leading to reduced serum immunoglobulin (Ig) levels and an abnormal antibody response (4). Although some associated gene defects have been recognised to cause a disruption in B-cell differentiation and B-cell function (ICOS, TACI, CD19, BAFF-R, MSH5, CD20 and CD81) (5-8), in the majority of patients no genetic defect has yet been established. Approximately one-half of CVID patients also show abnormalities in the T-cell compartment (9).

X-linked agammaglobulinaemia (XLA) is a hereditary immunodeficiency caused by mutations in the *BTK* gene (10), representing a tyrosine kinase that is important for B-cell development (11;12). Patients present with recurrent bacterial infections at a very young age and a profound deficiency of all Ig isotypes resulting from an arrest in B-lymphocyte development in the bone marrow. Other features are chronic and

unremitting systemic infections with enteroviruses (13;14), mycoplasma and ureaplasma as well as chronic gastroenteritis caused by rotaviruses and *Giardia lamblia* (11;15;16). Furthermore, a variety of malignancies have been reported, including lymphoreticular malignancies (16-20) and gastric and colorectal carcinoma. In a few families, other gene mutations have been recognised involved in B-cell development that cause autosomal recessive congenital agammaglobulinaemia.

Other, more frequent, antibody deficiencies are immunoglobulin G (IgG) subclass deficiency and selective antibody deficiency with normal immunoglobulins (SADNI). A clinically significant IgG subclass deficiency is defined as reduced levels of one or more IgG subclasses (IgG1–4) in a patient with normal total IgG concentrations (21) and is characterised by recurrent sinopulmonary infections and inadequate response to vaccination. IgG Subclass deficiency might merely be a laboratory finding in the absence of a clinical disorder; up to 20% of the population may have subnormal levels of one or more subclasses (22). In adults, the most common deficiency is IgG3, whereas in children it is IgG2 (23). SADNI is classified as recurrent sinopulmonary infections and an abnormal response to polysaccharide vaccination in the presence of normal antibody levels (24;25). The prevalence of SADNI in two studies was 5–10% in children over 4 years of age who were referred with recurrent infections (26;27) and 8% in adult patients with recurrent pneumonia (28).

Other primary antibody deficiencies are the hyper-IgM syndromes, IgA deficiency and selective IgM deficiency.

The mainstay of therapy for patients with primary antibody deficiency is the use of prophylactic antibiotics and/or Ig replacement therapy in order to reduce the infection rate and end-organ damage. The most important complication of recurrent respiratory infections in antibody deficiency is the development of bronchiectasis, which may lead to chronic pulmonary disease (CPD). Diagnostic and treatment delay has been related to higher morbidity and subsequent reduced pulmonary function (15;29-32). It is therefore important to establish the diagnosis early in order to initiate appropriate treatment and to prevent irreversible end-organ damage.

Immunoglobulin replacement therapy

2.1. Historical perspective

Human Ig therapy for antibody deficiency was initiated by Bruton following his description of the first case of XLA in 1952 (10). The initial route of Ig administration was intramuscular (IMIg). In the USA, human intravenous immunoglobulin (IVIg) was first licensed for primary antibody deficiencies in 1981. This product was a less painful alternative and allowed administration of much larger volumes with fewer side effects (33;34). Since that time, more purified and better tolerated IgG preparations have become available (33). At the same time, subcutaneous IgG (SCIg) therapy became available (35-37). Initially, SCIg infusion was limited by the (slow) infusion rate, although this has improved over the years (38-41). However, IVIg still remains the dominant form of Ig replacement therapy in the USA and Europe.

2.2. Production and content of immunoglobulins

Multiple safety steps are undertaken to provide a safe, pure and efficient product that contains antibodies against a wide range of pathogens. Many blood-borne pathogens, such as human immunodeficiency virus (HIV), hepatitis C virus, parvovirus B19, West Nile virus and prions, have been recognised to constitute a danger for patients treated with immunoglobulins. In the mid-1990s an outbreak of hepatitis C occurred in Europe and was associated with Ig therapy (42-45). Specific methods have been developed to assure maximal removal of pathogens (46), including donor screening for HIV and hepatitis B and C virus, detergent and solvent treatment, virus inactivation, destruction and removal steps such as pasteurisation at 60 °C, treatment with low pH/alcohol, and nanofiltration. However, a small risk of transmission of blood-borne diseases remains.

All available Ig products contain >95% IgG with all IgG subclasses represented. Most products contain no IgM and very small amounts of IgA. IgM is removed because it can rapidly form large complexes leading to a variety of adverse reactions. CVID patients frequently develop anti-IgA antibodies that may provoke anaphylactic reactions to IgA-containing blood products. Various strategies are used to remove

all traces of donor IgA molecules (47;48), and minor differences in IgA levels exist between the current products.

IgG1 and IgG2 make up 85% of the total amount of IVIg, whereas IgG3 and IgG4 are minor components (5–8% and 1–5%, respectively). The repertoire of immune antibodies is thought to reflect the infectiological experience of the donor population. To best cover the needs of patients, it is believed that Ig therapy is optimal when the recipient belongs to the same population as the donors (47;48).

2.3. Effect of IgG replacement therapy on infections and end-organ damage

2.3.1. Morbidity and mortality

The life expectancy of patients with XLA and CVID was very poor before the era of Ig replacement therapy. In 1971, the 10-year survival rate was 37% in 201 CVID patients treated with IMIg (49). Few, if any, XLA patients survived past early childhood before antibiotic and Ig therapy became available (50). In one study, ca. 75% of 170 XLA patients diagnosed before the introduction of IVIg had developed chronic lung disease at the age of 20 years, 5–10% had developed a cor pulmonale and 18% had died, mostly due to infectious complications (51). Owing to early diagnosis, more effective treatment with Ig and more liberal use of antibiotics, survival of patients with an antibody deficiency has significantly improved over the last decade (49;52). A study of 248 CVID patients receiving IVIg therapy reported a 10-year survival of 78% compared with 97% in the general population (3).

However, despite IgG therapy, patients with complications due to inflammatory autoimmune diseases and neoplasm's still have a shorter life expectancy (52). Diagnostic delay is a major concern and the main cause of the development of organ damage. In a 2005 review of 89 patients with a primary antibody deficiency, the median diagnostic delay was 2 years (mean 4.4 years), resulting in substantial morbidity (30). A moderate improvement had been achieved compared with an earlier 1980s study that showed a median delay of 5.5 years in adults and 2.5 years in children (50).

2.3.2. Benefit in acute respiratory infections

A strong body of evidence has demonstrated the efficacy of Ig therapy in CVID and XLA patients. The studies are listed in Table 1. Although sample sizes are small and most of the studies are retrospective case series, it is clear that Ig therapy reduces the incidence and severity of infections, the rate of hospitalisation and the use of antibiotics, albeit at variable doses and variable follow-up periods in these studies.

Scarce evidence supports Ig replacement in IgG subclass deficiency or SADNI patients. Ig replacement may be appropriate if prophylactic antibiotics do not result in fewer infections. In a retrospective study, patients with a selective or combined IgG subclass deficiency with four or more episodes of bacterial RTIs per year were treated with IVIg 0.4 mg/kg/month, which led to a 50% reduction in antibiotic-demanding (i.e. presumably bacterial) infections in 70% of patients ($P < 0.001$) (53). In an open-label study (54), 10 adult patients with symptomatic IgG subclass deficiency were treated with monthly IVIg for 1 year followed by 3 months of observation of IVIg therapy. All patients showed a significant reduction in the number of infections, days of antibiotic usage and hospitalisations during the 12 months of IVIg. The benefit of IgG replacement in patients with SADNI has not been evaluated in randomised, placebo-controlled trials, however, uncontrolled series of paediatric SADNI patients have consistently reported significant decreases in the number of infections (55).

2.3.3. Benefit in chronic respiratory disease and end-organ damage

Few studies have evaluated the effect of Ig treatment on the evolution of chronic sinopulmonary disease and pulmonary damage such as bronchiectasis. In a prospective study of 24 previously untreated adult CVID patients (56), the effect of IVIg on the evolution of lung damage was evaluated 2 years after stable trough levels of IgG > 6 g/L were achieved. To achieve the desired trough levels, patients with chronic pulmonary disease (CPD) needed higher doses of IVIg than those without CPD (285 ± 53 mg/kg/21 days vs. 222 ± 23 mg/kg/21 days; $P = 0.002$). Some pulmonary improvement was demonstrated in patients with CPD, as the forced expiratory volume in 1 s (FEV_1) as a percentage of the predicted value increased from 54 ± 13% (range

Table 1 Efficacy of immunoglobulin therapy

Year (reference)	Patients	Type of study	n=	Treatment regimen	Outcome
1979 (124)	Adults, children; CVID, XLA	RCT	20	IMIg 3.3 g/month vs. IVIg 150 mg/ kg/month	0,3 infections vs. 0,1 infections/month
1984 (88)	Adults, children; CVID, XLA	PC	21	IVIg 300 mg/kg/3 weeks vs. previous IMIg	Less days of illness/antibiotic use (p<0,1) for 18 of 21 patients, less sick days (total 834 days to 258 days), less days on antibiotics (total 3249 days to 1820 days)
1985 (89)	Adults; CVID, XLA	PC	7	IVIg 600 mg/kg/month vs. previous IMIg	Reduction in infection rate (25 vs. 4 /year)
1987 (125)	Children; antibody deficiency	CO	12	IVIg 150 vs. 500 mg/kg/ month cross-over	Significant reductions in days with infections
1990 (126)	Children; antibody deficiency	RCS	23	IVIg 150-300 mg/kg/3 weeks vs. previous IMIg	75% less days of fever/antibiotics, 91% less hospital days, 50% less absence from school, 65% less days with infection (p<0.01)
1992 (90)	Adults, children; XLA	RCS	20	(1) no treatment	Reduction in hospitalization (p< 0.01) and pneumonias (p<0.04) in (4) vs. (1) en (2)
			14	(2) IMIg< 100 mg/kg/3 weeks	
			3	(3) IVIg up to 200 mg/kg/3 weeks	
			15	(4) IVIg 350-600 mg/kg/ 3 weeks	
1999 (91)	Children; XLA	RCS	31	IVIg mean dose/ 3 wk 390 mg/kg	Reduction in annual bacterial infections (0.06 vs. 0.4) P<0.01
2001 (92)	Adults, children; CVID, XLA	RCT; CO	43	IVIg: Adults 300 vs. 600 mg/kg/ month,	Mean infection rate: low dose 3.5±2.6/patient vs. high dose 2.5±2.4/patient (p=0.004)
				Children 400 vs. 800 mg/kg/ month	Mean duration of infections: low dose 33 days vs. high dose 21 days (p=0.015)

Table 1 Continued

Year (reference)	Patients	Type of study	n=	Treatment regimen	Outcome
2001 (127)	Adults, children; CVID	RCS	19	IVIg 300-600 mg/kg/3 weeks, trough IgG> 4 g/L	0.28 vs. 0.16 RTI/patient/year (p<0.01)
2002 (128)	Adults, children; CVID	RCS	50	IVIg 300-400 mg/kg/3-4 weeks	Reduction in number of patients with pneumonia from 42 to 11 after treatment(p<0.01)
2004 (56)	Newly diagnosed adults; CVID	PC	24	IVIg 200-300 mg/kg/3-4 weeks	Serious infections[1] 1.3 ± 1.2/year to 0.2 ± 0.5/ year, p<0.01, mild infections[2] 4.9±4/year to 2.2±2.0/year p<0.01
2004 (93)	Adults, children; XLA	RCS	23	IVIg 300-400 mg/kg/3-4 weeks	Pneumonia/year 0.8 to 0.1, p<0.01, reduction of hospitalization (p=0.02) during therapy
2005 (129)	Adults; CVID	RCS	7	no treatment, then IVIg 200, then 400 mg/kg/3 weeks	Infections/patient-year 5.0 to 2.8, p<0.01 to 1.5, p=0.02 during therapy
2005 (94)	Children; CVID, XLA, HIM	RCS	46	IVIg median dose 370 mg/kg/2-4 weeks	Infection rate 12.4 to 3.2/patient/year; hospitalization rate 1.2 to 0.2/patient/year after treatment
2006 (130)	Adults, children; CVID, XLA	RCS	26	IVIg 400 mg/kg/3-4 weeks	Pneumonia in 80% to 35% of pts (p<0.01), hospitalization rate 88% to 46% (p<0.0025) after starting treatment

RCS= retrospective case series; RCT= randomized controlled trial; CO=crossover; PC=prospective cohort
XLA= X-linked agammaglobulinaemia; CVID= Common variable immunodeficiency
IVIg = intravenous immunoglobulin, IMIg= intramuscular immunoglobulin.
RTI= respiratory tract infections, n= number of patients.
[1] Serious infections denotes pneumonia, sepsis, meningitis and/or pulmonary abscess.
[2] Rate of mild infections denotes episodes of bronchitis, otitis, sinusitis or fever per year.

26–67%) to 61 ± 13% (range 35–76%) (*P* = 0.004) and overall high-resolution computed tomography scores improved in patients with CPD, which was attributable to the reduction in bronchiectasis and signs of inflammation. The median IgG trough level in the CPD group was 7.2 ± 1.4 g/L (range 5.7–9.8 g/L) and in the group without CPD it was 8.5 ± 1.6 g/L (range 6.0–11.6 g/L). A recent study (57) confirmed that CVID patients with bronchiectasis need higher replacement doses to achieve similar IgG trough levels (0.70 ± 0.29 g/kg/month vs. 0.53 ± 0.20 g/kg/month). In contrast, a prospective study in 22 patients receiving IVIg treatment showed progression of pulmonary changes in one-half of the patients after a 3-year follow-up (31). In another multicentre prospective study of 224 CVID patients (58), the number of patients with CPD and chronic sinusitis also increased over 11.5 years despite IVIg treatment and despite the significant reduction in the percentage of patients who were affected by acute respiratory infections.

In conclusion, the beneficial effect of Ig replacement therapy on short-term respiratory complications (acute respiratory infection rate and antibiotic usage) is undisputable, but conflicting evidence exists on the beneficial effect on long-term complications.

2.3.4. Benefit in gastrointestinal disease

Non-infectious inflammatory disease of the gut is common in patients with primary antibody deficiencies. Many of the inflammatory disorders mimic the classic forms of the disease (in the absence of immunodeficiency) such as coeliac disease, inflammatory bowel disease and pernicious anaemia (3;50;59-62), but they differ in the pathogenesis and are unresponsive to Ig treatment. Different explanations may justify the occurrence of these conditions in patients with antibody deficiencies despite appropriate Ig replacement therapy. First, IVIg therapy only substitutes IgG, whilst IgA (the major secretory antibody at mucosal surfaces) is not replaced. Other possible explanations could be on-going inflammation after treated infections (63) and concurrent T-cell defects (3;63).

Currently, treatment for gastrointestinal disease associated with antibody deficiencies is based on treatment modalities used for similar disorders in immunocompetent patients (64-66).

2.4. Indications for IgG replacement therapy

The most obvious justification for Ig therapy is the absence of functionally mature B-cells, as in XLA patients. Another clear ground for IgG replacement is reduced levels of serum Ig in patients with recurrent bacterial infections.

In general, Ig replacement therapy is indicated in (i) patients with IgG levels <2 g/L, (ii) patients with documented frequent infections and a specific antibody deficiency with IgG levels between 2 g/L and 5 g/L or (iii) patients with IgG levels >5 g/L but severe and recurrent infections and a specific antibody deficiency (67).

In asymptomatic individuals with IgG subclass deficiencies, Ig replacement therapy is not warranted. It is advisable to follow these patients, as some might evolve into CVID (68). Patients with an IgG subclass deficiency or SADNI who suffer from severe or recurrent RTIs despite prophylactic antibiotics are candidates for Ig therapy (53;54).

Although scarce evidence exists, treatment of patients with an IgG subclass deficiency and/or poor response to polysaccharide vaccines might include vaccination with pneumococcal conjugate vaccine (69). IgG replacement might be appropriate for selected SADNI patients with recurrent infections despite immunisation with conjugate vaccines and appropriate antibiotic treatment. Additional grounds for IgG replacement might be uncontrollable recurrent otitis media with risk for permanent hearing loss, the presence of bronchiectasis, and patients with hypersensitivity to multiple antibiotics.

Finally, management of other conditions predisposing to recurrent sinopulmonary infections such as asthma and allergic rhinitis is warranted.

2.5. Choice of product and administration route

The efficacy and safety of IVIg (67;70;71) and SCIg (41;72;73) has been well established. Both treatment options appear to be safe, with comparative efficacy and costs. Two small comparative studies showed no significant difference in the rate of infections, adverse events (74) or IgG steady-state levels (41) between the two treatment modalities.

The benefits of weekly SCIg infusions over 3-weekly IVIg therapy include

stable IgG levels, less frequent and less severe systemic side effects (35;38-40;73), no necessity for venous access and more flexibility in the patient's social life. A disadvantage of SCIg is the limitation in volume that can be administered, prompting weekly infusions.
Many centres provide training in SCIg and IVIg home therapy, which has clear benefits for patients, such as increased lifestyle flexibility and taking control of the management of their disease (75-77).
The choice of product must be individualised for each patient and will be based on the clinical condition of the patient, the patient's wishes and the side effects. Patient-related factors that influence this choice are age, cardiovascular impairment, renal dysfunction, thromboembolic risk, and the presence of (pre) diabetes mellitus and anti-IgA antibodies. The product features that affect clinical tolerability are listed in Table 2.

Table 2 Product features affecting clinical tolerability (131)

• Volume load (rate of infusion)
• Osmolality
• Sodium content
• Sugar content
• IgA content

The difference in tolerance to the various Ig products in individual patients is striking and unpredictable and is probably caused by the spectrum and concentration of antibodies and other plasma proteins. For elderly patients and patients with congestive heart failure, concentrated IgG products (10%) and products with a lower sodium content might be more suitable (78). Sodium content has also been associated with a higher incidence of thromboembolic complications and could thus be a further restricting factor (79). Various sugars have been added to the different IgG products (sorbitol, glucose, sucrose or maltose) to minimise IgG aggregate formation. Although concentrations are not particularly high, they may lead to deregulation of glucose levels in diabetic patients. Sucrose has also been associated with renal failure due to osmotic nephritis (80). Risk factors associated

with renal adverse events include pre-existing renal disease, diabetes, hypovolaemia, sepsis, age (≥65 years) and concomitant nephrotoxic therapy (79).

The therapeutic strategy of IgG replacement in patients with anti-IgA antibodies is a critical issue. It has been demonstrated that patients with IgA antibodies can be treated more safely with SCIg (81;82). SCIg might induce tolerance by gradual exposure to IgA owing to the slow resorption of the subcutaneous deposit of Ig (83).

2.6. Dosing regimen and trough levels

The major goal of Ig replacement therapy in patients with primary antibody deficiency is to reduce and prevent morbidity, such as infection rate and end-organ damage, and mortality.

The appropriate dose of Ig for antibody-deficient patients is determined by the IgG trough level, the median half-life of IgG and the intrinsic metabolism of the patient. However, the pharmacokinetics of IVIg shows considerable intrapersonal and interpersonal variability, and the patient's intrinsic IgG production will interfere with measurements of half-life and clearance.

It has been demonstrated that serum IgG levels initially decline rapidly following intravenous infusion and by day 7 a substantial part of the infused Ig has disappeared (84;85), followed by a period of more gradual decline to baseline depending on the metabolic rate of the patient and the half-life of the preparation. Using radio labelled IgG it has been shown that the catabolism of IgG follows multi-compartmental first-order kinetics. After an initial period of equilibrium between intravascular and extravascular compartments, the concentration of IgG in the serum is eliminated at a rate independent of the remaining concentration (86).

The mean half-life of IgG is 25–32 days in patients with a primary immunodeficiency, however, in patients with extremely low baseline levels of IgG the variations in half-life are greater (84;87).

In contrast to IVIg, weekly subcutaneous infusions of IgG will generate a local depot resulting in slow absorption and a nearly constant serum level of IgG.

Ig dosing is more complex in patients in whom IgG production is deficient but not completely absent such as in CVID, subclass deficiency or SADNI. In patients with high baseline serum IgG concentrations (>5 g/L), the half-life of IgG tends to be the longest, suggesting that intrinsic IgG production might prolong the calculated half-life of IgG, leading to an incorrect estimate of the half-life (86).

Another important parameter in determining the dose of replacement therapy is the clinical condition of the patient in relation to the IgG trough level.

Several studies have compared the effect of dosage and IgG trough levels in patients with a primary humoral immunodeficiency (see Table 3). These studies are difficult to compare as they are heterogeneous with regard to methodology, routes of administration (IMIg, SCIg and IVIg) and study populations. Furthermore, the studies are limited by sample size and follow-up. None the less, the majority of these studies show that higher Ig dosage and IgG trough levels result in fewer infections and a reduced duration of the remaining disease episode (88-96). The exact IgG trough level that will protect antibody-deficient patients against recurrent bacterial infection and progression to chronic lung damage remains uncertain. Long-term outcome parameters such as structural organ damage are difficult to follow; IgG trough levels are thus used as surrogate parameters. The majority of studies show a significant reduction in infection rates with a higher IgG trough level, especially >8 g/L (92). A recent meta-analysis showed that the incidence of pneumonia declined by 27% with each 1 g/L increment in IgG trough level. The incidence of pneumonia with a trough level of 5 g/L was 0.113 cases/patient-year versus 0.023 cases/patient-year with a trough level of 10 g/L (97).

Furthermore, a recent study supports the idea of individualising the replacement dose. In this prospective cohort study, 107 CVID and XLA patients showed a wide range of IgG trough levels preventive of breakthrough bacterial infections (5–17 g/L) with a replacement dose ranging from 0.2 g/kg/month to 1.2 g/kg/month (57).

Table 3 Effect IgG trough level on infections outcome in primary antibody deficiency

Year (ref)	Patients	type of study	n=	treatment regimen	outcome
1984 (88)	Adults, children; CVID, XLA	PC	21	IVIg 300 mg/kg/3 weeks vs. IMIg	Average IgG levels increased 2,4 g/L
1984 (95)	Adults, children; CVID, XLA	RCT	16	IVIg 100 mg/kg/month	Trough levels increases with higher dose
			19	IVIg 400 mg/kg/month	
1985 (89)	Adults, children; CVID, XLA	PCS	7	IVIg 600 mg/kg/month vs. IMIg 100 mg/kg/2-4 weeks	IgG trough levels 5-7.5 g/L: 4 vs. 25 hospital admissions, improvement sinusitis/ bronchiectasis
1987 (96)	Adults, children; CVID, XLA	RCT CO	12	IVIg 600 vs. 200 mg/kg/month	Trough levels> 5 g/L with fewer minor (12 vs. 31)and major infections (3 vs. 16)
1992 (90)	Adults, children; XLA	RCS	20	no treatment	IgG trough levels 0.9± 0.8 vs. 2.2±0.8 vs. 2.8± 0.7 vs. 6.5± 1.2 g/L
			14	IMIg< 100 mg/kg/3 weeks	
			3	IVIg up to 200 mg/kg/3 weeks	
			15	IVIg 350-600 mg/kg/3 weeks	
1999 (91)	Children; XLA	RCS	31	Mean dose 390 mg/kg/3 weeks	Annual incidence of bacterial infections by trough level: 0 when >8 g/L, 0.05 when 5-8 g/L, 0.16 when <5 g/L
2001 (92)	Adults, children; CVID, XLA	RCT CO	43	IVIg: Adults 300 vs. 600 mg/kg/ month Children 400 vs. 800 mg/kg/ month	Trough level 6.4 vs. 9.4 g/L; mean infection rate: low dose 3.5±2.6/patient vs. high dose 2.5±2.4/patient (p=0.004); mean duration of infections: low dose 33 days vs. high dose 21 days (p=0.015)
2005 (94)	Children; CVID, XLA and HIM	RCS	46	IVIg median dose 370 mg/kg/2-4 weeks	Infection rate less with trough levels >5g/l vs. 3 vs. <3 g/L (p<0.01)

RCS= retrospective case series; RCT= randomized controlled trial; CO=crossover; PC=prospective cohort; PCS=prospective case series, IVIg = intravenous immunoglobulin, IMIg= intramuscular immunoglobulin, n= number of patients.
XLA= X linked agammaglobulinaemia; CVID= Common variable immunodeficiency; HIM= hyper IgM.

Based on various studies, a 2006 review by members of the Primary Immunodeficiency Committee of the American Academy of Allergy, Asthma and Immunology (98) recommends that IgG trough levels should be >5 g/L for patients with agammaglobulinaemia and 3 g/L greater than the initial IgG level for patients with CVID. Furthermore, an IgG trough level of >5 g/L reduces the infection rate, and IgG trough levels >8 g/L might improve chronic pulmonary outcome (88-96).
IVIg is administered every 3–4 weeks with a usual starting dose of 0.4 g/kg for patients without chronic lung disease and 0.6 g/kg for patients with bronchiectasis. SCIg dosing is the same but divided over 4 weeks. Given the costs involved, leading factors in choosing the dosing regimen are the clinical parameters of an individual patient in combination with trough levels.
Dosage adjustments are required in exceptional situations such as acute illness, before/after surgery and pregnancy (99). No specific protocols for pregnant women have been published. Replacement therapy is not only necessary for the mother but also for the foetus. The foetus and the newborn synthesis little Ig and rely on active placental transport of IgG from the maternal circulation (99-104). During pregnancy, the increase in blood volume can cause inadequate IgG trough levels, which may lead to an increased infection rate. IgG trough levels should be checked more often during pregnancy and breastfeeding to make sure that they remain adequate, and the patient must be informed about the importance of these measures. A study from 2001 showed that normal maternal IgG and IgG subclass concentrations can also be achieved by the SCIg route (105).

2.7. Risks and adverse reactions of immunoglobulin therapy

2.7.1. Intravenous immunoglobulin

Although in general IVIg is well tolerated by patients with an antibody deficiency, side effects can occur at any point during treatment and are mostly related to the infusion rate. Patients who are naïve to IgG replacement or who have active infections have an increased risk of infusion-related adverse effects. These effects may be related, in part, to the formation of antigen–antibody complexes (67). Factors that

potentially affect the risk and intensity of adverse events include age and underlying conditions, such as migraine and cardiovascular or renal disease.

Infusion-related adverse events can be immediate (during the infusion), delayed (hours to days after the infusion) or late reactions. Immediate reactions can be either true IgE-mediated anaphylaxis or 'anaphylactic' reactions. The difference is that the latter is associated with hypertension rather than hypotension. True anaphylaxis may occur in patients who are deficient in IgA but still have the capacity to produce IgE (106).

The most common reaction is an immediate adverse event related to the infusion rate. Mild reactions include headache, flushing, chills, fever, nausea, anxiety and muscle aches. Moderate reactions consist of chest pain, wheezing and vomiting, and severe reactions are severe headaches, chest pain and wheezing. Slowing or temporarily stopping the infusion may allow the symptoms to subside. Infusion can then be continued at the previously tolerated rate. If this fails to prevent symptoms, pre-medication with antipyretics, antihistamines and/or corticosteroids may help to treat the symptoms. When symptoms persist or rapidly worsen, immediate discontinuation of the infusion and administration of adrenaline may be warranted (67).

Overall, the risk of infusion-related events has been reduced owing to improved manufacturing processes. In a large prospective study of 459 antibody-deficient patients with 13.508 infusions, the reaction rate was 0.8% over 2 years (111 events, comprising 91 mild and 20 moderate); no severe reactions occurred (0.1% were moderate and 0.6% were mild). The most important symptoms were headaches, chills and fever and most of the reactions occurred during higher infusion rates. Most reactions occurred in patients with an active infection (5.1%) (107).

In another retrospective study (71 patients, 1231 infusions), 152 adverse events (12.3%) occurred in 35 patients, of which 131 events were mild (86.2%) and no severe reactions occurred. Again, most adverse events were related to the infusion rate and active infections (108).

The most common immediate side effect of IVIg therapy is a headache that may last for several days. The reported incidence was as high as

56% in one study (109). Often, patients who experience headaches during infusion have a history of migraine or hypertension. In the latter it may be prevented by taking an extra dose of blood pressure medication before the infusion. Non-steroidal anti-inflammatory drugs (NSAIDs) may also be effective in case of minor headaches. It is proposed that headaches can be prevented by slowing the infusion rate (110) or reducing the total dose of IVIg (111). In general, many patients develop headaches only during the first few cycles of IVIg therapy (111).

Urticarial reactions are common during IVIg and might be minimised by pre-medication with antihistamine or low-dose corticosteroids. A low-grade fever often occurs during infusion, which can be prevented by antipyretics.

Delayed symptoms consist of nausea, malaise and myalgia and typically occur 1–3 days after administration of IVIg.

Late adverse events are rare but can be severe and unpredictable as they can occur after months or years of uneventful therapy. They have mainly been reported with the higher dosing regimens used for treatment of autoimmune and haematological disorders. The most important late side effects are acute renal failure and thromboembolic events (112). Other late adverse events include aseptic meningitis, stroke (113), progressive neurodegeneration (114), neutropenia, autoimmune haemolytic anaemia, skin reactions and (rarely) arthritis and pseudohyponatremia. A thorough and complete medical evaluation of each patient is warranted before initiation of therapy to identify risk factors associated with severe side effects. Less than 5% of the reported cases of IVIg-associated renal insufficiency occurred in patients with primary immune deficiency (115). Acute renal failure is usually oliguric and reversible and has been related to osmotic injury secondary to sucrose. The majority of cases of renal dysfunction occurred in the first 10 days after the first cycle of IVIg therapy (116-118). Patient risk factors associated with renal adverse events include pre-existing renal disease, diabetes mellitus, hypovolaemia, sepsis, age (≥65 years) and concomitant nephrotoxic therapy (79).

2.7.2. Subcutaneous immunoglobulin

Serious systemic adverse events are rare in subcutaneous therapy. Common reactions due to SCIg therapy are local swelling, redness and an itching or burning sensation, occurring in 8–49% of infusions. These effects are rarely serious and disappear after several hours and are more common at initiation of treatment (41;73;74). The safety of SCIg has been established in a study with 165 primary antibody-deficient patients (40); 106 adverse systemic reactions were recorded during 33.168 subcutaneous infusions in 28 patients, of which 100 were mild and 6 were moderate. No severe or anaphylactic reactions occurred. In a randomised crossover trial of IVIg and SCIg treatment, the systemic reaction rate of IVIg therapy was 5% compared with a SCIg reaction rate of 3.3% (74). However, most studies have reported <1% systemic events in SCIg therapy (119).

Additional therapies

Infections in IgG-treated patients might indicate inadequate dosing and IgG trough levels. Patients who continue to have respiratory infections and develop CPD despite adequate IgG trough levels should be treated more aggressively by a strategy directed against the ongoing process of inflammation and infection, such as prophylactic antibiotics (120;121), macrolides (also as anti-inflammatory agents) (122), corticosteroid inhalation therapy (123), bronchodilators, mucolytic agents, and physical or mechanical aids for airway clearance. Serial sputum testing, including antibiotic sensitivity testing of the cultured organism, should direct prophylactic antibiotic therapy.

Conclusion

The primary antibody deficiency syndromes are characterised by an inability to produce clinically effective Ig responses. Patients most commonly present with recurrent respiratory infections. Diagnostic delay remains common owing to limited awareness of the presenting features. Diagnostic delay and subsequent delay in initiation of Ig replacement therapy can result in increased morbidity and mortality.

Replacement therapy with Ig increases life expectancy and reduces the frequency and severity of infections. However, the effect on end-organ damage remains disputable.

Clear indications for IVIg replacement are the absence of functionally mature B-cells such as in patients with XLA, and, secondly, reduced levels of serum Ig in patients with recurrent bacterial infections such as CVID patients. In general, IgG replacement is indicated in (i) all patients with IgG levels <2 g/L, (ii) patients with documented frequent infections and a specific antibody deficiency with IgG levels between 2 g/L and 5 g/L and (iii) patients with IgG levels >5 g/L but severe and recurrent infections combined with a specific antibody deficiency (67).

Both IVIg and SCIg treatment appear to be safe, with comparable efficacy. Infusion-related adverse reactions have been reduced considerably in recent years owing to improved manufacturing processes. The advantages of SCIg are more stable IgG levels, the absence of serious systemic adverse events and more flexibility in the patient's social life.

The overall consensus is that (i) the starting dose of IVIg and SCIg should be 400 mg/kg/month and 100 mg/week, respectively, (ii) IgG trough levels should be >5 g/L for patients with agammaglobulinaemia and 3 g/L greater than the initial IgG level for patients with CVID and, finally, (iii) the clinical response should be foremost in choosing the right dose and trough level.

Key areas for further research would be to determine the optimal dose of Ig therapy required to improve overall health outcome; to develop infusion methods leading to improved and less frequent SCIg dosing (e.g. once in 2 or 3 weeks); and identification of prognostic markers to allow specific intervention and optimal therapy for subgroups of patients.

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3

The spectrum of disease manifestations in patients with common variable immunodeficiency and partial antibody deficiency in a university hospital

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Abstract

Background

Common variable immunodeficiency (CVID) represents a heterogeneous disease spectrum that includes recurrent infections and complications such as autoimmunity, inflammatory organ disease and an increased risk of cancer. A diagnostic delay is common in CVID patients.

Purpose

To determine the spectrum of clinical manifestations, immunological characteristics, and the time to diagnosis of 61 adult CVID and 18 patients with a partial antibody deficiency (SADNI and IgG subclass deficiency).

Methods

A retrospective cohort study was performed in patients who met the ESID/PAGID for CVID, IgG subclass deficiency and SADNI. Medical records were reviewed to obtain patient demographics, clinical and laboratory data.

Results

Infections were the main presentation of all antibody deficient patients and the number of patients with infections declined during IgG therapy. The development of bronchiectasis continued despite IgG therapy, as well as the development of auto inflammatory conditions. Non-infectious disease complications were present in 30% of CVID patients at the time of diagnosis and this increased to 51% during follow up despite IgG therapy. The most common complications were autoimmunity or lymphoproliferative disease. The median time to diagnosis was 10 years and in the patients with non-infectious complications the time to diagnosis was considerably longer when compared to the group of patients without complications (17.6 vs. 10.2 years, p=0.026).

Conclusion

In contrast to the partial antibody deficiencies we found a considerable delay in the diagnosis of CVID, especially in those patients who were dominated by non-infectious complications, and thus increased awareness would be beneficial. Pulmonary and other complications may continue despite adequate IgG replacement therapy suggesting other causes responsible for these complications.

Introduction

Primary antibody deficiencies represent the largest group of primary immunodeficiencies and are characterized by B-cell dysfunction (1). Common variable immunodeficiency (CVID) is the most heterogeneous group among the antibody deficiency syndromes and the estimated prevalence is 1: 25.000 (2). The typical clinical presentation of CVID is that of recurrent bacterial respiratory tract infections but other infections may also occur. Previous gene defects have found to be associated with the CVID phenotype (ICOS, TACI, CD19, BAFF-R, MSH5, CD20 and CD81) (3-6), however, less than 10% presents within families. In most patients symptoms will start after puberty, and the diagnosis is often made when the patient has already reached adulthood. A diagnostic delay is quite common with a mean of 6–8 years after the onset of symptoms (7;8), it can however take longer than a decade before a patient is diagnosed (9). The diagnostic delay and subsequent delay in (immunoglobulin) therapy is thought to be a major cause of the development of organ damage resulting in increased morbidity and mortality (8-12). Pulmonary damage is the most frequent complication and may result from recurrent infections and/or immune dysregulation. Other complications due to the underlying immune dysregulation (7;9;13) include lymphoproliferative disease (granulomatous disease (14), lymphadenopathy and hepatosplenomegaly), autoimmune disease (15), gastrointestinal disease such as chronic inflammation (16) and an increased risk of cancer (17;18). While a substantial part of the CVID patients remain relatively free of such problems, a subgroup will develop one or more of these disease related complications. Attempts have been made to classify the heterogeneous CVID population into more similar subgroups by using clinical parameters (9) and flow cytrometric markers of B-(13;19;20) and T cells (21;22) in order to define parameters that might predict which patients will be particularly prone to these complications.

Clinically less severe and much more common antibody deficiencies are immunoglobulin G (IgG) subclass deficiency and selective antibody deficiency with normal immunoglobulins (SADNI) that are mainly characterized by recurrent sinopulmonary infections. In 20%

of cases however, the finding of lower IgG subclass levels is merely a laboratory finding that does not necessarily lead to symptoms (23). Nevertheless, these conditions occasionally progress to CVID (23;24). In the current retrospective study we determined the spectrum of clinical manifestations during the long-term follow-up of adult CVID patients in the outpatient clinic of our university hospital. For comparison we also included patients with IgG subclass deficiency or SADNI. We studied the clinical features, the immunological characteristics and the time to diagnosis according to the previous presented classification systems (9;13).

Methods

The department of internal medicine and Infectious diseases at the University Medical Centre Utrecht in The Netherlands serves as a referral center for adult patients with primary immunodeficiencies. We performed a retrospective cohort study of all patients with an antibody deficiency that met the European Society for immune deficiencies (ESID)/Pan-American Group criteria for Immunodeficiency (PAGID) (25) for Common Variable Immunodeficiency (CVID), immunoglobulin G (IgG)- subclass deficiency and selective antibody deficiency with normal immunoglobulins (SADNI) that have attended our outpatient clinic between 1978 and 2011. SADNI was defined as a failure to produce antibodies to polysaccharide vaccines. The response to 23-valent pneumococcal polysaccharide vaccine was assessed 4 to 6 weeks after vaccination and evaluated according to age and vaccination history. If the adult patient was not previously vaccinated with a conjugated pneumococcal vaccine, the IgG response to the 23 valent polysaccharide vaccine was found abnormal when less than 8 more of the 11 measured antibody titres had reached a value of $\geq$1.0 µg/ml. Patients with a hyper IgM syndrome, XLA or congenital agammaglobulinaemia were excluded from this study because of the distinct entity and so where patients with a secondary hypogammaglobulinaemia due to protein loss, drugs, malignancy, or infection.

All data entries were cross-checked by an independent physician.

Clinical follow up

Medical records from the patients were reviewed to obtain patient demographics, clinical manifestations and laboratory data. Clinically stable patients had usually visited with a frequency of once or twice a year at our outpatient clinic. Routine physical examination together with standard laboratory measurements and IgG trough levels were performed in CVID patients once or twice a year. Until recently a pulmonary function test, High Resolution Computed Tomography (HRCT), abdominal ultrasound and a gastro- and colonoscopy were performed only in case of symptoms, this policy has changed over the last 2 years.

The time to diagnosis was defined as the time in years between the year of onset of disease-related symptoms (infectious or non-infectious complications as depicted below) and the year of diagnosis. Patients that still had ongoing infections after the start of IgG therapy were scored when antibiotics were prescribed or when positive bacterial culture results were obtained in combination with a clinical diagnosis. To assess the onset of disease we used the following clinical criteria based on the PID warning signs issued by the ESID (26): 1) $\geq$ 4 new middle ear infections within 1 year; 2) $\geq$ 2 or more sinus infections within 1 year; 3) $\geq$ 2months on antibiotics with little effect; 4) Two or more pneumonias within 1 year or recurrent pneumonias; 5) Recurrent, deep skin or organ abscesses; 6) Persistent thrush in mouth or fungal infection on skin; 7) Need for intravenous antibiotics to clear infections; 8) Two or more deep-seated infections including septicaemia (i.e. osteomyelitis, meningitis, severe pneumonia, and arthritis); 9) Gastrointestinal infections with Giardia Lamblia or Campylobacter.

All immunologic data was entered twice in the database by different persons.

Disease complications

Symptomatic chronic pulmonary disease was defined as chronic obstructive pulmonary disease or asthma, bronchiectasis or inflammatory pulmonary conditions (such as interstitial lung disease). Other complications related to immune dysregulation have been previously

categorized into five phenotype categories (9): (1) lymphoproliferative disease, (2) autoimmune disease, (3) gastrointestinal disease (4) malignancies or (5) no disease related complications. Patients were scored according to these categories (i.e. 1 complication = 1 category) and individual patients were scored for having one or more complications.
Autoimmunity included cytopenias (chronic autoimmune hemolytic anemia, chronic autoimmune thrombocytopenia, and unexplained leucocytopenia) and organ-specific autoimmunity (rheumatoid arthritis and systemic lupus erythematosus meeting the American Rheumatism Association criteria, Graves' disease, pernicious anemia and atrophic gastritis (biopsy proven) and alopecia areata). Lymphoproliferative conditions were defined as unexplained persistent lymphadenopath (onpalpitation,ultrasoundorcomputertomographyscan),granulomatous disease (biopsy proven) or hepatosplenomegaly (ultrasound proven). Gastrointestinal disease was defined as gastrointestinal symptoms combined with biopsy proven endoscopic abnormalities and included Helicobacter pylori positive gastritis, inflammatory colitis, malabsorption with villous atrophy, polyps and adenoma. Malignancies were defined as biopsy proven lymphoid, bone marrow or solid organ cancer.

Laboratory data

Immunoglobulin titres, T- and B cell phenotyping and in vitro mitogenic and antigenic T cell proliferation responses had been performed in most patients**,** between 2007 and 2011. The timing was aimed just before the administration of immunoglobulins. At the time of these measurements only one patient used prednisolone 10 mg/day on a chronic basis. IgG trough levels had been measured once or twice a year in clinically stable patients during follow up, and more often in patients with disease-related complications. In our current daily practice we aim to reach IgG through levels of at least 8 g/L (27).
CVID patients were classified according to the classification of EURO Class trial(13), a classification scheme based on flowcytometric B cell phenotyping and the clinical course of the patient. The T- and B cell populations were analyzed by four-color flow cytometry using whole blood and antibodies to CD3, CD45, CD27, CD4, CD8, HLA-DR, CD38,

CD45RA and CD19, CD27, CD38, CD10, IgM, IgG, IgA, IgD, respectively, as described previously(28;29).

For the T- and B lymphocyte functional assays peripheral blood mononuclear cells (PBMC) were obtained and the following stimuli were supplemented: phytohemagglutinin, Concanavalin A, tetanus toxoid, purified protein derivative (PPD), Candida albicans and diphtheria toxin. Assay conditions were verified by a control sample run in parallel. The percentage of response was defined by the number of positive responses to a stimulus divided by the total number of tests. For B cell differentiation assays, PBMC were cultured with either pokeweed mitogen or Staphylococcus aureus antigen and IL-2 (28).

Statistical analysis

Statistical analyses were performed using Mann–Whitney U tests and Pearson's chi square tests with SPSS 15.0 for Windows. A P value of 0.05 or less was considered significant.

Results

Sixty-one CVID patients, nine IgG subclass deficiency patients and nine patients with Selective antibody deficiency with normal immunoglobulins (SADNI) were analyzed. All patients had been diagnosed between 1978 and 2010. The age at onset of symptoms could be traced in 55 of 61 CVID patients, the year of diagnosis was known for all patients.

Common variable immunodeficiency

The baseline characteristics are shown in table 1. The median age of the 58 CVID patients that were still alive at the time of analysis was 38 years (IQR 26-58 yrs). Of all patients that were analyzed 36 were female (59%) and 25 male (41%) and the vast majority of patients were Caucasian (56 patients, 92%). The recorded follow up in our hospital since diagnosis ranged between 4 and 13 years (median 7 yrs). The median age at which CVID related symptoms had started was 17 years (IQR 4-23 yrs) and the median age at diagnosis had been 27 years (IQR 14-43 yrs). The median time to diagnosis had been ten years (IQR 5-16 yrs), which is addressed in further detail below.

Table 1 Baseline characteristics

	CVID (n=61)	**IgG subclass deficiency** (n=9)	**SADNI** (n=9)
Sex, number of pt (%)			
female	36 (59%)	7 (78%)	6 (67%)
male	25 (41%)	2 (22%)	3 (33%)
Ethnicity, number of pt (%)			
Caucasian	56 (92%)	8 (89%)	9 (100%)
middle East	2 (3%)		
Far east	1 (2%)	1 (11%)	
mix	2 (3%)		
Current age[1]	38 (26-58)	42 (25-49)	45 (26-53)
Age at start symptoms[1]	17 (4-23)	16 (4-38)	33 (20-45)
Age at diagnosis[1]	27 (14-43)	38 (14-45)	44 (21-52)
Time to diagnosis[1]	10 (5-16)	4 (1-24)	2 (1-10)
Follow up since diagnosis[1]	7 (4-13)	3 (1-12)	1 (1-3)
Follow up since start therapy[1]	6,5 (3-13)	3 (1-11)	1 (0-2,5)
Death, number of pt	3	0	0
Causes of death	pneumonia, brain abcess sepsis with pneumonia		
Number of patients with family members* with a confirmed antibody deficiency	6 (in 3 families)	0	0
Number of pt with renal failure	3	0	0

Pt: patients; CVID:Common variable immunodeficiency; SADNI: Selective antibody deficiency with normal immunoglobulins, n= number of patients.
* First or second degree family members; [1] Median years (interquartile rang)

The majority of CVID patients (42 patients, 69%) already had related symptoms before the age of 20 years, however, only 36% had been diagnosed before the age of 20 suggesting a substantial time to diagnosis (Figure 1). Notably, two patients had developed symptoms after the age of 60 years.

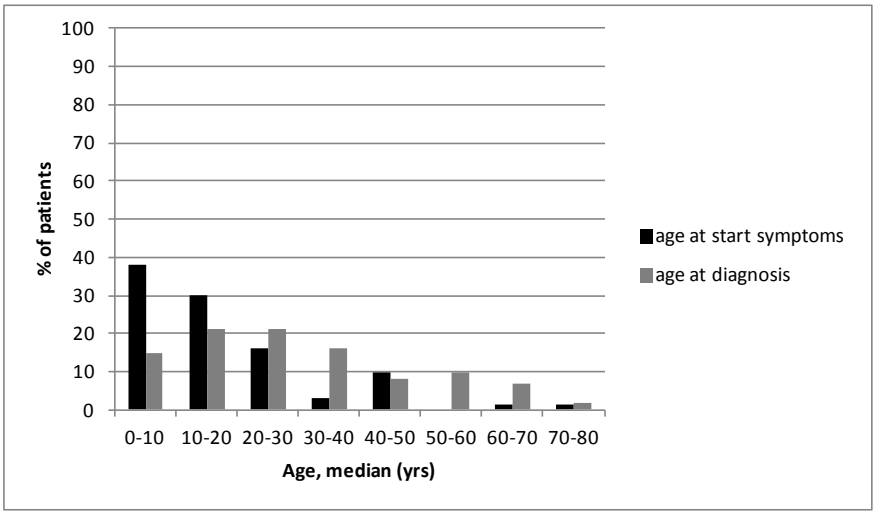

Figure 1 Age at onset symptoms and at diagnosis of CVID in retrospective analysis.
yrs = years

In all CVID patients intravenous (n= 45) or subcutaneous (n= 16) IgG substitution therapy had been started. The vast majority had commenced this therapy within one year after the diagnosis had been established (54 patients, 88%). All patients had been started on immunoglobulin therapy in our hospital, either in the department of paediatrics or in the adult department and follow up of patients took place in our hospital. Currently, it is standard to start with immunoglobulin dosing of 0.4 g/kg. The dosage of patients differs and had been adjusted according to IgG trough levels (aim > 8.0 g/l) and clinical response. The median IgG trough level in the last decade was 8.8 g/L. Twenty-six patients (43%) also received antibiotic prophylaxis at any point during follow up.

Infections

Before immunoglobulin therapy 90% (n=55) of CVID patients had suffered from (recurrent) respiratory infections as shown in Table 2. Four patients (7%) had suffered from severe herpes virus infections (Varicella and/or Herpes simplex) before diagnosis. Infections of the urogenital tract, central nervous system, gastro-intestinal tract and skin were much less common. Four patients (7%) had no recurrent or

severe infections before the diagnosis and this remained so thereafter: two patients had arthralgia at the time, one was screened because of a CVID sibling, and the last patient was screened for chronic non-infectious diarrhea.

The median IgG trough level of the patients with infections after start of IgG therapy was not significantly different in comparison to the patients without infections (9.2 g/L vs. 8.7 g/L, respectively). Although eight of the 55 patients (14 %) with respiratory infections became free of infections after the initiation of IgG therapy the majority of patients still suffered from respiratory infections (47 of 55 patients, 85%; Table 2), however these appeared to be less frequent. Figure 2 shows the reduction in

Table 2 Number of patients with infections prior to diagnosis

	CVID (n=61) Number of patients (%)	**IgG subclass deficiency** (n=9) Number of patients (%)	**SADNI** (n=9) Number of patients (%)
None	4 (7%)	0	0
Recurrent respiratory infections	56 (93%)	9 (100%)	9 (100%)
URTI	43 (71%)	3 (33%)	6 (67%)
LRTI	31 (51%)	6 (67%)	5 (56%)
Gastrointestinal infection[1]	7 (12%)	0	0
recurrent	6/7 (86%)	NA	NA
Other infections			
abdominal abcess	1		1
skin infections	3	1	
herpes virus infection	4		
hepatitis	1		
meningitis	3	2	1
pancarditis	1		
urinary tract infection	5	1	
panuveitis (toxoplasma)	1		

CVID: Common variable immunodeficiency; SADNI= Selective antibody deficiency with normal immunoglobulins. URTI: upper respiratory tract infection, LRTI: lower respiratory tract infection, n= number of patients.

[1] Gastrointestinal infections: Giardia Lambliae, Campylobacter enteritis, Salmonella enteritis.

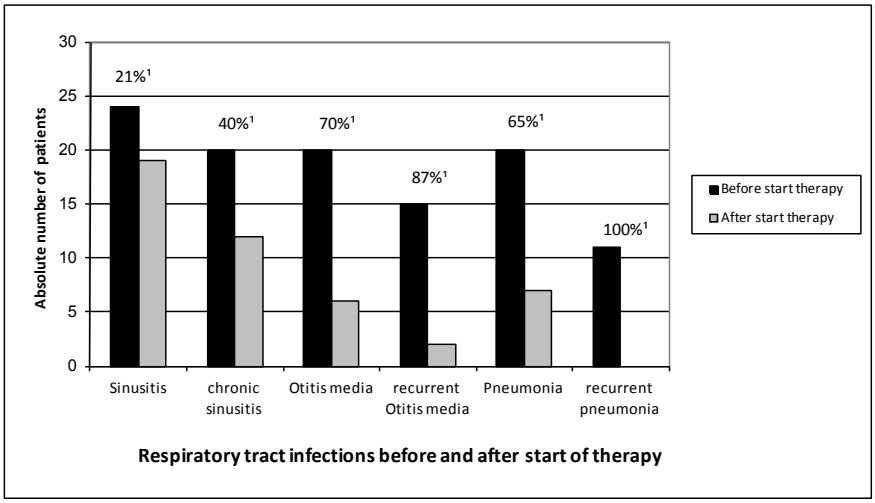

Figure 2 Number of CVID patients with respiratory tract infections before and after start of immunoglobulin therapy.

[1] % decline number of patients with respiratory tract infections

the number of patients with respiratory tract infections following the institution of immunoglobulin therapy. The most prominent reduction was established in middle ear infections and pneumonia (70-100% reduction; Figure 2). However, least effect was accomplished in the occurrence of sinusitis: 79% of patients with sinusitis prior to IgG therapy still suffered from one or more episodes and 60% of patients with chronic sinusitis were not cured.

Seven (11%) patients had suffered from gastrointestinal infections before diagnosis of which 4 with Giardia Lamblia, eight more had a gastrointestinal infection (13%) after start of therapy. During follow up one patient was diagnosed with Progressive Multifocal Leukoencephalopathy (PML) during prednisolone treatment for interstitial pulmonary disease and one patient with CMV colitis.

Pulmonary disease and chronic sinusitis

Symptomatic chronic pulmonary diseases were diagnosed in 20 (33 %) CVID patients before the start of therapy and this number increased to 34 (56%) patients after the start of immunoglobulin therapy (Table 3). Before the start of therapy the majority had been diagnosed with asthma (13 of 20 patients) and none during follow-up. Chest CT

Table 3 Symptomatic chronic lung disease in 61 CVID patients.

	Before start of therapy	After start of therapy
	Number of patients (%)	Number of patients (%)
Symptomatic chronic pulmonary disease*	20/61 (33%)	34/61 (56%)
Structural airway changes (bronchiectasis)	2/20 (10%)	10/34 (29%)
Interstitial lung disease	6/20 (30%)	9/34 (26%)
granulomatous disease	3/6 (50%)	3/9 (33%)
fibrosis	3/6 (50%)	6/9 (67%)
COPD/asthma	13/20 (65%)	16/34 (47%)

*Symptomatic chronic lung disease was defined as chronic obstructive pulmonary disease (COPD) or asthma, complications due to infections (e.g. bronchiectasis) and auto inflammatory pulmonary conditions such as interstitial lung disease.

scanning demonstrated the presence of bronchiectasis in two patients at diagnosis and in another eight during the follow-up, which is likely to be an underestimation since only 12 patients underwent chest CT scanning at or before diagnosis. Of the eight patients diagnosed with bronchiectasis during follow up only two patients had median IgG trough levels <8 g/L. Another three patients developed interstitial lung disease during follow up. Chronic sinusitis was present in 20 patients (33%) and responded in eight patients to IgG therapy.

Other disease related complications

Table 4 displays the number and nature of CVID related complications and the different disease complications observed before diagnosis and during follow up. Despite immunoglobulin therapy patients still developed complications during follow up.

At diagnosis 18 patients (29%) had one (14 patients) or more complications (4 patients) and this number increased to 31 patients (51%) during follow up. Of the 18 patients who already had one or more complication at diagnosis seven patients developed additional complications of a different etiology. Additional or new complications developed in 20 patients (33%) during follow-up. Of more than half

Table 4 CVID related complications before and after start of immunoglobulin therapy in 61 CVID patients.

	Before start of therapy	**After start of therapy**
	Number of patients (%)	Number of patients (%)
Complications		
Yes	18 (30%)	31 (51%)
No	43 (70%)	30 (49%)
Number of complications/patient		
0	43 (70%)	30 (49%)
1	14 (23%)	18 (29,5%)
2	4 (6.5%)	9 (15%)
3	0	4 (6.5%)
4	0	0
Type of disease [1, 2]		
Lymphoproliverative	8/61 (13%)	17/61 (28%)
granulomatous disease	4	8
lymphadenopathy	4	11
hepatosplenomegaly	4	11
spleen	2	8
liver	1	1
both spleen and liver	1	2
Autoimmune disease	10/61 (16%)	14/61 (23%)
non-septic arthritis	2	2
autoimmune cytopenia	3*	9
organ related	2**	3**
alopecia	3	3
Malignancy	0	4/61 (7%)
anal	0	1
thyroid	0	1
seminoma	0	1
Bladder	0	1
Gastrointestinal disease	4/61 (6,5%)	13/61 (21%)
oesophagitis		2
gastritis	1	7
villous atrophy	1	5
inflammation ileum/colon/rectum	2	8
angiodysplasy		1
polyps/adenoma	1	4
Malignancy		1
Nodular lymphoid hyperplasia		6

Table 4 Continued.

	Before start of therapy	After start of therapy
	Number of patients (%)	Number of patients (%)
Number of patients with phenotypes [2]		
None	43 (70%)	30 (49%)
Lymphoproliverative	4 (7%)	5 (8%)
Autoimmune	9 (15%)	8 (13%)
gastrointestinal disease	1 (1.5%)	4 (6,5%)
Malignancy	0	1 (1,5%)
Lymphoproliverative and autoimmunity	1 (1.5%)	3 (5%)
Lymphoproliverative and gastrointestinal disease	3 (5%)	5 (8%)
Lymphoproliverative and malignancy	0	1 (1,5%)
Autoimmune, malignancy and gastrointestinal disease	0	1 (1,5%)
Lymphoproliverative, autoimmune and gastrointestinal disease	0	2 (3%)
Lymphoproliverative, malignancy and gastrointestinal disease	0	1 (1,5%)

[1] patients can have more than 1 condition. [2] according to chapel et al 2008
*Of the three patients with cytopenia, one male suffered from autoimmune hemolytic anemia and two females from Idiopathic thrombocytopenic purpura.
**Before diagnosis of CVID: one patient was diagnosed with diabetes mellitus and another patient with systemic lupus erythematodes. After diagnosis one patient was diagnosed with hypothyroidism.

of the patients that developed new complications during follow-up (18 of 31; 58%) these complications could be categorized into one single category. Lymphoproliferative and autoimmune complications were the most frequent complications that were already present at the time of diagnosis. Newly diagnosed complications during follow up were mostly of a lymphoproliferative or gastrointestinal nature. Splenomegaly was fairly uncommon in our cohort at diagnosis (only three patients at diagnosis and another six during follow-up) which is likely an underestimation since abdominal ultra sound was not performed routinely. None of the patients had been diagnosed with cancer before the diagnosis of CVID and during follow-up four patients developed a malignancy (anal carcinoma at the age of 27, thyroid cancer at the age of 22, seminoma at the age of 58 and bladder cancer at the age of 61). Three of these patients also had other complications

as a result of extended immune dysregulation (table 4). All three patients who developed end stage organ failure during follow up were diagnosed with terminal renal insufficiency and are currently on hemodialysis. In two patients renal insufficiency was caused due to underlying vascular problems and one patient was diagnosed with interstitial nephritis.

The IgG trough levels of the patients that had developed new complications since IgG substitution did not differ significantly compared to the patients that had not developed new complications.

Time to diagnosis

The median time to diagnosis of CVID was substantial, 10 years (IQR 5-16 yrs) compared to 2.5 years (IQR 1-18 yrs), p=0.016 of the partial antibody deficiencies (IgG subclass deficiency and SADNI taken together). Two patients with bronchiectasis at diagnosis had a diagnostic delay as long as 24 and 32 years, respectively. Figure 3 shows the age at the onset of symptoms and the age at the time of CVID diagnosis as well as the time to diagnosis in relation to the number of complications. Patients with one or two complications had been significant older at the time the CVID diagnosis was made compared to the patients without complications. (n=17 vs. 44, 39 yrs vs. 28 yrs, p=0.03) The median time to diagnosis in the group of patients with non-infectious complications was seven years longer in comparison to the group of patients without these disease complications (10.2 vs. 17.6 years, P=0.026). Patients with autoimmune disease (10 patients) had a median diagnostic delay of 17 years (IQR 0-39 yrs). Especially alopecia had been present long before the diagnosis of CVID was made (median 25 years, range 11-39 yrs). All four patients with granulomatous disease had a relative long time to diagnosis (respectively 32, 21, 16 and 7 years). Of the four patients (6.5%) with gastrointestinal disease diagnosed before the diagnosis of CVID, the diagnostic delay was 32, 10, 7 and 5 years respectively. The diagnostic delay of the three patients that had died during follow up had been 28, 14 and 12 years, respectively.

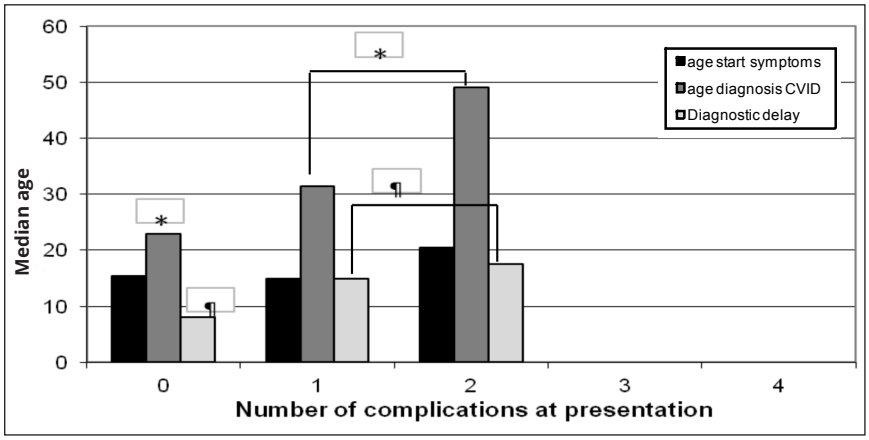

Figure 3 Median age at start of symptoms, at diagnosis and diagnostic delay of CVID patients compared to the number of CVID related non-infectious complications at diagnosis.
* and ¶: patients without any complication vs. patients with one or more complication: p<0.05.

Mortality

Three patients had died during follow-up. One male patient had been diagnosed with CVID fourteen years after his symptoms started at the age of 63 years. He also suffered from cardiovascular disease and diabetes mellitus and died at the age of 70 years as the result of pneumonia. A female patient died at the age of 31 years due to a brain abscess. She had been diagnosed with CVID at the age of 27 after suffering from upper respiratory tract infections, Herpes Zoster infections and lymphoproliferative disease for 12 years (since the age of 15). The third patient, also a female, died at the age of 49 due to a sepsis of unknown cause. She had been diagnosed at the age of 45 years but had suffered from numerous clinical problems years before that (since the age of 17 years).

Laboratory evaluation

The median IgG of all CVID patients at diagnosis was 3.8 g/L (IQR 2.1-4.9 g/L). (Table 5) Patients who were diagnosed with lymphoproliferative conditions, autoimmune disease and gastrointestinal disease had a lower IgG at diagnosis compared to those without complications. (2.0 g/L (IQR 1.2-3.6) p=0.02; 2.8 g/L (IQR 1.6-4.4) p=0.03; 1.5 g/L (IQR 0.63-2.9) p= 0.002 vs. 4.5 g/L (IQR 2.8-5.2) respectively).

Table 5 Immunological parameters of CVID patients.

	(n=61)	**Females** (n=36)	**Males** (n=25)
Ig levels at diagnosis (g/L)	Median (range)	Median (range)	Median (range)
IgG (7,0-16,0)	4,0 (2,3-5,1)	4,0 (2,4-5,0)	3,9 (1,12-5,3)
IgA (0,7-4,0)	0,1 (0,0-0,6)	0,3 (0,0-0,66)	0,04 (0,2-0,42)
IgM (0,4-2,3)	0,4 (0,22-0,9)	0,5 ((0,26-1,04)	0,31 (0,2-0,43)
Number of patients (%) with lymphocyte below lower limit counts[1]	**Number of patients (%)**	**Median (range)**	**Number of patients with complications**
CD3+ T cells [2]	10/52 (19,2%)	(376-684 cells/ul)	9/10
CD4+ T cells [2]	12/54 (22,2%)	(55-344 cells/ul)	9/12
CD8+ T cells [2]	5/52 (9.6%)	(37-155 cells/ul)	5/5
Inverted CD4/CD8 ratio [2]			
under lower limit	10/53 (18.7%)	(0.30-0.90)	5/10
above upper limit	7/53 (13.2%)	(3.5-9.0)	4/7

[1] absolute lymphocyte counts per cubic millimeter
[2] reference values: CD3+ T cells (100-400), CD4+ T cells (400-1300), CD8+ T cells (200-700), CD4/CD8 ratio (1,1-3,2).
n= number of patients

B cell phenotype

During routine clinical evaluation flowcytometric B-cell phenotyping had been performed in 46 patients, and 70% of these patients had normal numbers of total CD19 positive B cells. Patients with complications related to immune dysregulation had lower absolute numbers of CD19 positive B lymphocytes then those that did not (median 256/mm³ (IQR 189-384/mm³) vs. 111/mm³ (IQR 39-308/mm³), p=0.007). Furthermore, we established significant differences in the absolute numbers of cells in the B cell subsets between patients with and without complications. (Table 6) According to the EURO class classification(13), two patients (4%) had less than 1% of CD19+ B cells of lymphocytes of which one patient had been diagnosed with an autoimmune complication and the other patient with a lymphoproliferative condition and gastrointestinal disease. Patients with ≥ 1% B cells of total lymphocytes were further divided into two categories based on the percentage of class-switched memory B cells deficiency (≤2% or >2% of the circulating B cell pool). The percentage of patients with ≤2% of class switched memory B cells

was 18% (11 of 46 patients). Seven of these 11 patients (63%) had one or more non-infectious complications. Patients with complications and >2% of class switched memory B cells had lower median numbers of class switched memory B cells then patients without complications. (11.2/mm³ (IQR 6.6-23.2/mm³) vs. 3.6/mm³ (IQR 0.5-10.7/mm³), p=0.013).

Table 6 shows the median numbers within the B cell compartment and each different complication. Low numbers of switched memory B cells was associated with autoimmune and lymphoproliferative disease. Furthermore patients with splenomegaly and granulomatous disease had lower median numbers of switched memory B cells vs. patients without these conditions. (This is not shown in table 6: 9.8/mm³ (IQR 4.6-23/mm³) vs. 0.4/mm³ (IQR 0.1-3.5/mm³), p=0.001, 1.5/mm³ (IQR 0-10/mm³) vs. 11.5/mm³ (IQR 7-23/ mm³) p=0.016 respectively). Figure 4 shows the relation between the number of complications and the B cell subsets. Although not significant, there seems to be a trend of a decrease in the absolute numbers of naive B cells, as the number of complications increases. Patients with one or more complication had significant lower naïve B cells (158 vs. 71.5 cells/ mm³ p=0.04) and IgG memory B cells (7 vs. 1.5 cells/ mm³ p=0.01).

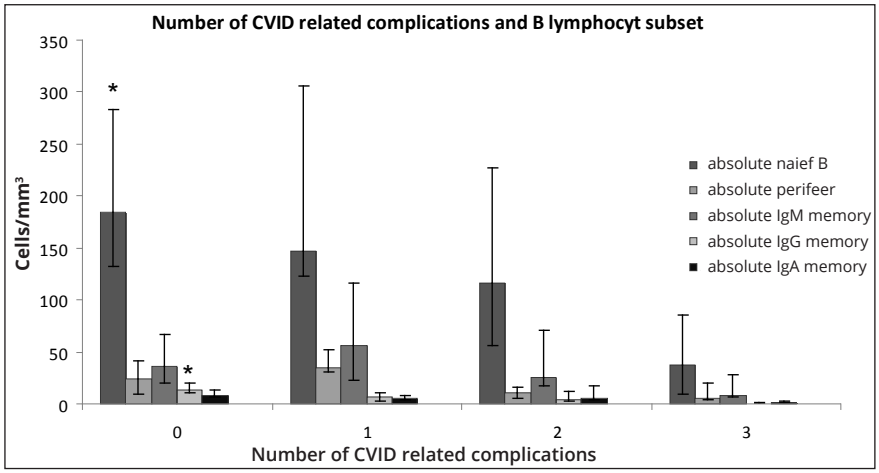

Figure 4 Total number of CVID related complications and B lymphocyte subset.
* Patients with complications had significant lower number of absolute naief B cells and IgG memory B cells vs. patients without complications, p<0.05.

Table 6 Median absolute numbers of B lymphocyte subset in CVID patients with and without complications

	Total B cells [1,3]	Naive B cells [1, 3]	RBE [1, 2, 3]	IgM memory B cells [1, 3]
No complications (n=24)	256 (189-384)	158 (105-257)	19.5 (5-37)	30 (14-62)
Complications				
Total (n=28)	111 (39-308)*	71.5 (37-201)*	11.5 (5-23)	12 (2-61)
Lymphoproliverative (n=16)	110.5 (16-308)*	65.5 (31-208)*	10.5 (2-18)	15.5 (2-71)
Auto immune(n=13)	79 (29-139)**	60 (39-114)*	11.5 (4-21)	8.5 (2-28)*
Gastrointestinal disease (n=13)	79 (22-322)*	79 (35-212)	11 (2-27)	22 (1-64)

	IgG memory B cells [1, 3]	IgA memory B cell [1, 3]	Class switched memory B cells [1, 3]
No complications (n=24)	7 (4-13)	3.5 (2-9)	11.5 (7-23)
Complications			
Total (n=28)	1.5 (0-6)**	2 (0-6)	3.5 (0.2-11)*
Lymphoproliverative (n=16)	1 (0-7)*	2 (0-10)	3.5 (0-12)*
Auto immune(n=13)	1 (0-2)*	1.5 (0-3)*	3 (0-5)*
Gastrointestinal disease (n=13)	1 (0-11)*	3 (0-9)	4 (1-26)

CVID: common variable immunodeficiency; n= number of patients.
Complications: Lymphoproliverative-, auto immun- and gastrointestinal disease. Malignancies excluded.
* <0,05 and **<0,001, comparison is made between the group of patients with and without complications
[1] Median absolute numbers/mm³ (interquartile range)
[2] Recent bone marrow immigrants B cells
[3] CD19+ B cell compartment:
naive B cells: IgM+IgD+CD27-CD10-, recent bone-marrow emigrants (RBE): IgD+CD10+CD38++,
non-Ig class-switched IgM+IgD+CD27+ memory B cells, IgG+CD27+ and IgA+CD27+ memory B cells.
Reference values, (all in absolute numbers): From: van Gent et al, Clinical Immunology (2009) 133, 95-107
CD19+ B lymphocytes (100-400); Naief B cells (72-257); recent bone marrow emigrants B cells (6-41);
IgM memory B cells (10-39); IgG memory B cells (2-51); IgA memory B cells (1-20).
Class switched memory B cells (2-40)

T cell phenotype

T lymphocyte abnormalities were present in almost 20% of CVID patients (Table 5) and the majority of patients with decreased numbers of CD3, CD4 and CD8 positive T cells had been diagnosed with one or more complication, most often lymphoproliferative and autoimmune diseases. Table 7 shows the median absolute numbers within the T cell compartment in patients with and without complications. Patients with one or more complications had significant lower numbers of CD3 and CD4 positive T cells. Furthermore patients with autoimmune disease had significant lower absolute numbers of CD3, CD4 and CD8 positive T cells compared to patients without complications. Absolute numbers of naive CD4+ T cells and naive CD8+ T cells were significant lower in the group of patients with any complication (Table 7).

Figure 5 shows the relation between the number of complications and T lymphocytes subsets. The absolute number of CD3 + and CD4+ T lymphocytes was significantly lower in the group of patients with one or more complication. All 50 patients that were tested on lymphocyte proliferation had normal responses on mitogens PHA, ConA and PWM (48 patients were tested) and only a few patients responded abnormal on Candida (41/45 patients, 2%), PPD (5/43 patients, (12%), tetanus toxoid (3/44 patients, 7%) and diphtheria toxin (6/40 patients, 15%).

Partial antibody deficiency

Of the nine patients with IgG subclass deficiency most were Caucasian (8 patients, 89%) and one patient originated from the Far East. Seven patients were female (78%) and two male (22%). The median age when symptoms started was 16 years (IQR 4-38 yrs) and the median age at diagnosis was 38 years (IQR 14-45 yrs). None of the patients died during follow up. Four patients were diagnosed with an IgG4 deficiency of which one had IgG therapy and one antibiotic prophylaxis. Two patients had an IgG2 deficiency, one on IgG therapy and antibiotics prophylaxis as well and one patient was on antibiotics only. Two patients had a IgG3 deficiency, both on antibiotic prophylaxis and one patient with a combined IgG2, 3 and 4 deficiency who was treated by IgG therapy and antibiotics prophylaxis. The two patients without therapy became

Table 7 Median absolute numbers of T lymphocyte subset in CVID patients with and without complications

	CD3+ T cell [1, 2]	CD4+ T cell [1, 2]	CD8+ T cell [1, 2]	Activated CD4+ T cell [1, 2]	Naive CD4+ T cell [1, 2]
No complications (n=24)	1450 (1050-1899)	812 (607-1131)	533 (372-738)	8 (3-17)	367 (223-580)
Complications					
Total (n=28)	968 (671-1649)*	572 (330-989)*	379 (222-619)	12 (6-24)	121 (52-390)*
Lymphoproliverative (n=16)	792 (643-1589)*	566 (357-1021)	270 (151-597)*	18 (9-27)	115 (14-406)*
Auto immune (n=13)	806 (1050-1899)*	512 (261-578)*	335 (224-562)*	11 (5-29)	84 (43-221)*
Gastrointestinal disease n=13)	975 (560-1604)*	601 (419-989)	311 (153-558)*	11 (2-24)	99 (21-269)*
	Eff/Mem CD4+ T cell [1, 2]	**Activated CD8+ T cell [1, 2]**	**Naive CD8+ T cell [1, 2]**	**Eff/Mem CD8+ T cell [1, 2]**	
No complications (n=24)	418 (306-548)	9 (3-16)	308 (214-357)	233 (147-363)	
Complications					
Total (n=28)	412 (250-569)	14 (5-25)	99 (43-156)**	237 (120-429)**	
Lymphoproliverative (n=16)	507 (164-584)	20 (7-41)*	86 (30-158)**	141 (106-392)*	
Auto immune (n=13)	336 (244-435)	11 (6-18)	84 (40-135)*	217 (120-495)*	
Gastrointestinal disease (n=13)	513 (156-613)	7 (3-23)	64 (29-125)**	181 (106-345)*	

CVID: common variable immunodeficiency; n= number of patients.
Complications: Lymphoproliverative-, Auto immun- and gastrointestinal disease. Malignancies excluded.
*<0.05 and **<0.001
[1] Median absolute numbers/mm³ (interquartile range)
[2] CD3+ Tcell compartment:
CD3+ T cells were divided into CD4+ and CD8+ subsets, and subsequently into CD38+HLA-DR+ activated T cells, CD45RA+CD27+ naïve, and non-naïve CD45RA+CD27-, CD45RA-CD27+ or CD45RA-CD27- T cells.
Reference values, (all in absolute numbers): From: van Gent et al, Clinical Immunology (2009) 133, 95-107
CD3+ T lymphocytes (100–400); CD4 T+ lymphocytes (400–1300); CD8+ T lymphocytes(200–700); CD4/CD8 ratio (1.1–3.2);
Activated CD4+ T lymphocytes (2.5-8.5); Naive CD4+ T lymphocytes (240-790); Effector/memory CD4+ T lymphocytes (150-500);
Activated CD8+ T lymphocytes (4-19);Naive CD8+T lymphocytes (220-400); Effector/memory CD8+ T lymphocytes (50-190);

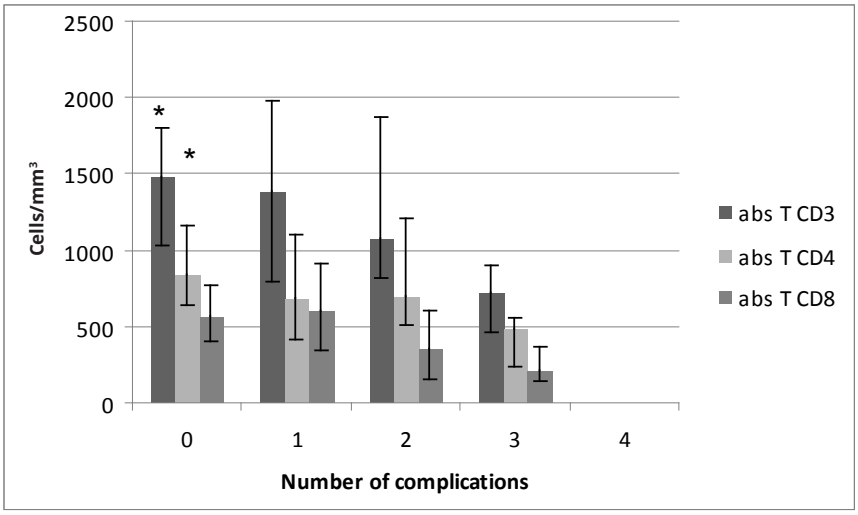

Figure 5 Total number of CVID related complications and T lymphocyte subset.
* Patients with complications had significant lower number of CD3 and CD4 vs. patients without complications, p<0.05.

free of infections. One out of four patients on antibiotic prophylaxes and one on out of three patients on IgG therapy became infection free. As shown in table 2 all patients had infections prior to diagnosis which mainly consisted of respiratory tract infections. Two of the 9 patients with an IgG subclass deficiency had suffered form from bacterial meningitis. In contrast to CVID, patients with a selective IgG deficiency did not suffer from gastrointestinal infections.

Despite a median time to diagnosis of 10 years (IQR 0-33 yrs) none of the patients developed chronic pulmonary disease or other complications during follow up.

Of the nine patients diagnosed with selective antibody deficiency with normal immunoglobulins (SADNI) six were female (67%) and three male (33%) and all were Caucasian. The median age at start of the symptoms was 33 years (IQR 20-45 yrs) and the median age at diagnosis was 44 years (IQR 21-52 yrs). The median IgG at diagnosis was 10.3 g/L (IQR 6.6-17.7 g/L). None of the patients died during follow up.

Two patients were treated by immunoglobulin substitution and four patients by prophylactic antibiotics. All patients had infections (mainly

respiratory tract) prior to diagnosis. Four patients without therapy, one of four patients on prophylactic antibiotics and one out of two patients on IgG therapy became free of infections. Two patients had chronic pulmonary disease at the time of diagnosis which is uncommon in SADNI patients of which one patient was diagnosed with SADNI because of the presence of bronchiectasis. This patient was started on immunoglobulin therapy and experienced fewer infections thereafter. The other patient was diagnosed with fibrosis on High resolution CT scanning of the lungs diagnosed after an episode with a complicated pneumonia. The median diagnostic delay was 6.5 years (IQR 1-30 yrs). Of the two patients with chronic pulmonary disease, only the patient with bronchiectasis had a considerable diagnostic delay (33 yrs).

Table 8 shows the decline in infections before and after diagnosis in the group of patients with an IgG subclass deficiency (n=9) or SADNI (n=9). Immunophenotyping was performed in seven of the nine IgG subclass patients and in three of the nine SADNI patients. The median absolute numbers of B cells were normal in both groups (IgG subclass deficiency 360/mm³ (IQR 170-583/ mm³), SADNI 201/ mm³ (IQR 165-484/ mm³). Within the B cell compartment of IgG subclass patients the

Table 8 Number of patients with infections before and after diagnosis in the group of patients with an IgG subclass deficiency (n=9) or selective antibody disorder (SADNI, n=9) [1]

	Before diagnosis PAD Number of patients (%)		**After diagnosis PAD** Number of patients (%)	
	IgG subclass deficiency	**SADNI**	**IgG Subclass deficiency ¶**	**SADNI #**
Sinusitis	1 (11%)	3 (33%)	2 (22%)	2 (22%)
Otitis media	1 (11%)	1 (11%)	1 (11%)	1 (11%)
Pneumonia	5 (56%)	3 (33%)	1 (11%)	0
Other [2]	4 (44%)	2 (22%)	2 (22%)	0

PAD: primary antibody deficiency
SADNI: Selective antibody deficiency with normal immunoglobulins
[1] Patients can have more than 1 condition
[2] IgG subclass deficieny: hidradenitis, meningitis, urine tract infection. SADNI: mastitis, abdominla abcess, meningitis.
¶ 2 patients without therapy, 4 patients on antibiotic prophylaxis, 1 patient on IgG therapy, 2 patients on IgG therapy and antibiotic prophylaxis.
# 4 patients without therapy, 3 patients on antibiotic prophylaxis, 1 patient on IgG therapy, 1 patient on IgG therapy and antibiotic prophylaxis.

absolute median numbers of IgM memory B cells were elevated (IgM memory B cells: 54.5/ mm³, IQR 20-111/ mm³, ref values: 20 (10-39/ mm³). The B cell compartment of SADNI patients showed a decreased absolute median numbers of naïve B cells and an elevated number of IgM memory B cells (naïve B cells: 61/ mm³ IQR 54-315/ mm³, ref values: 153/ mm³ (72-257/ mm³) and IgM memory B cells: 46/ mm³ IQR 43-56/ mm³, ref values: 20 (10-39/ mm³).

Discussion

CVID is the most common and most diverse primary antibody deficiency. It consists of a heterogeneous group of patients with variable infectious and immunological manifestations. CVID patients share clinical features but differ in their clinical course probably due to different underlying immunopathogenic mechanisms which are largely unknown as is the genetic cause.

This study presents the clinical and immunological data on a cohort of 61 patients. We found that the initial clinical presentation in our cohort was comparable to other cohort studies that have been published in the past (7;30;31). It has been reported that 90% of CVID patients suffer from one or more episodes of lower respiratory tract infections prior to diagnosis (32) and our findings were compatible to that. Because of the retrospective nature of the study the exact number of infections per year could not be traced and we were only able to distinguish patients that still suffered from infections from those that did not. When these two groups were compared, median trough levels did not differ significantly. This might be due to the fact that in our clinic we aim for an IgG trough level above 8 g/L for every patient and to the fact that this cohort is too small to find differences in IgG trough levels. The overall percentage of patients affected by new respiratory infections decreased during follow-up in our cohort. However, the effect on chronic sinusitis and acute episodes of sinusitis was less impressive. Previous reports have shown that immunoglobulin therapy is effective in preventing acute respiratory infections (27;30;33-36). Structural airway changes (e.g. bronchiectasis and bronchial wall thickening), parenchymal and interstitial lesions and chronic sinusitis are common

in adult CVID patients (38–79%) (12;32;37-42). Despite the considerable time to diagnosis in our cohort the prevalence of symptomatic chronic pulmonary disease and chronic sinusitis at diagnosis was lower in compared to other reports (18-40%, (12;31;32;43;44) and 36-90% (31;45) respectively). In our group only 10% of the patients with chronic pulmonary disease were found to have bronchiectasis prior to diagnosis of CVID, this increased to 29% with a median follow up of 7 years (range 4-13 yrs). However, this is likely an underestimation as high resolution computed tomography (HRCT) has not been applied for every patient at diagnosis and during follow up. Progression to chronic pulmonary disease continued in our cohort, despite IgG replacement therapy with adequate IgG trough levels (>8 g/L) which seems to be in accordance with previous reports (31;43;46-48). The pathogenesis of chronic pulmonary disease include recurrent respiratory tract infections but also non-infectious inflammatory conditions caused by immune dysregulation such as granulomatous and interstitial pulmonary disease (49). Immunoglobulin therapy has proven to be effective in preventing acute respiratory infections but conflicting data exist on the beneficiary effect on chronic pulmonary damage (12;31;43;46-48). Therefore the contribution of immune dysregulation to chronic pulmonary disease cannot be underestimated stressing the importance of early detection and directed therapy for chronic lung disease.

Chapel et al defined 5 distinct clinical phenotypes (9): patients with no complications, with autoimmunity, with polyclonal lymphocytic infiltration, with gastrointestinal disease or with malignancy. In our patient cohort 29% of patients had one or more complications at diagnosis, which increased to 51% of patients during follow up and despite immunoglobulin therapy. Compatible to other cohort studies (7;9) the most common complications were autoimmune and lymphoproliferative disease. Chapel et al described a similar frequency of 33% and 22% respectively (9). The increase was applicable for all categories of complications, however it was most prominent in gastrointestinal disease (6.5% to 21%), described earlier (31). Nevertheless, we established a lower prevalence of symptomatic

gastrointestinal disease compared with other reports (7;31). Furthermore only 10% had chronic diarrhea compared to 20-60% chronic diarrhea in other reports from the literature (7;16;50;51). The progression of chronic gastrointestinal diseases may still occur in patients with a primary antibody deficiency since IgG substitution will only substitute IgG, while IgA and IgM, the major secretory antibodies at mucosal surfaces are not replaced, and secondly, immune dysregulation and T cell abnormalities may contribute to gastrointestinal disease. Cytopenia was the most frequently diagnosed autoimmune disease. Three patients developed alopecia areata before diagnosis of CVID while alopecia in CVID has been described only in case reports (52-54). Malignancies are more common in CVID patients at a younger age, especially gastrointestinal cancer and lymphoma (17;18;31). In our cohort four patients were diagnosed with cancer of which two at a really young age (thyroid cancer (22 yrs) and anal carcinoma (27 yrs)). Pulmonary and other complications continue despite adequate replacement pointing at other causes responsible for this complication. Failure to diagnose CVID and therefore delaying the start of adequate therapy for specific conditions can cause considerable morbidity, particularly in case of progressive airway disease. The median time to diagnosis for the CVID patients in our cohort (10 years) was comparable to previously reported (3-15 years) (7;9;10;31;44;55). In a registry of nearly 400 patients from the United Kingdom, Germany, Sweden and the Czech republic, 20% of patients were diagnosed more than 15 years after onset of symptoms(9). In our study the median time to diagnosis in the group of patients with one complication or more was seven years longer ($p<0.05$) in comparison to the group of patients without complications. Although IgG substitution therapy seemed to have little effect on the development of these or new complications, reducing the diagnostic delay is essential in order to reduce infection-related complications such as pulmonary damage and chronic sinusitis. It must be stressed that the age at which symptoms had started is not a reliable parameter because of three reasons; first it is a retrospective estimation and second it cannot be calculated in months and third variation might even be one or two years.

Different classification schemes using clinical parameters(9), flow cytometric markers of B-(13;19;20) and T cells (21;22) have been proposed in order to subdivide the heterogeneous CVID population into more homogenous groups which might yield clues for possible pathogenic mechanisms as well lead to a model to predict which patients are prone to complications. Recently the first genome-wide association study in patients with CVID was performed which uncovered multiple novel susceptibility loci for CVID confirming the polygenic nature. Nevertheless these results could provide new mechanistic insights into immunopathogenesis (56).

The EURO class trial (13) established an association between a reduction in class switched memory B cells (≤ 2%) and CVID related complications. A reduction in class switched memory B cells (≤ 2%) was associated with a higher risk for splenomegaly and granulomatous disease. In other studies this association between a reduction of peripheral switched memory B cells and other clinical complications was confirmed (19;20;57). In our study, most patients had been diagnosed long before the bloodsampling. However, it is generally believed that the values in T and B cell phenotyping are more or less stable during the lifecycle of the patient. To our knowledge no data is published on this subject, it has only been confirmed by word of mouth during scientific sessions. The overall incidence of patients with a low percentage (≤2%) of class switched memory B cells was low. However, the association between autoimmune disease and a low percentage (≤2%) of class switched memory B cells was established as well as the association with splenomegaly and granulomatous disease. Furthermore patients with complications and >2% of class switched memory B cells had lower median numbers of class switched memory B cells compared to patients without complications. Therefore, patients with complications were more affected in their number of class switched memory B cells than patients without any complicating disease. Also, patients with complications related to immune dysregulation had lower absolute numbers of CD19 positive B lymphocytes then those that did not described by Yong et al (58) in children. Subsequently, we found a correlation between CD3+ T cells, CD4+ T cells, naive CD4+ and CD8+ T

cells and specific complications as previous described in the literature (21). Few studies have investigated the correlation with clinical features (21;22;59). One study found that a low count of absolute naive CD4+ T cells was associated with splenomegaly and autoimmunity. It is likely that T cells play an important role in the pathogenesis of auto inflammatory conditions in CVID (60-63). According to some studies the T-cell dysregulation such as the decrease in naive CD4+ T cells in certain patients could be due to abnormal thymus function (21;61), however accelerate T cell turnover as a result of the high infectious burden may also be an explanation (21).

In comparison, IgG subclass deficiency and SADNI patients did not develop any complications during follow-up as described in the sparse previous studies (64-66). The effect of therapy (IgG therapy or antibiotic prophylaxis) in these patients was most prominent on the occurrences of pneumonia. Although a decline in the number of patients with infections occurred in the group of partial antibody deficiencies, no distinction could be made between patients on or off therapy. Data on the immunological and clinical profile of immunoglobulin subclass deficiency are sparse in the literature. It appears that patients with IgG1 and/or IgG3 deficiency are more likely to have chronic and recurrent infections of the lower airways, while those with IgG2 and/or IgG4 deficiency are more likely to suffer from sinusitis and otitis media (67). Interestingly, in our cohort two of 9 patients had suffered from bacterial meningitis, which to our knowledge has not been described in earlier publications.

In conclusion, in our study the spectrum of illness for patients with CVID is in concordance with previous reports with predominantly respiratory tract infections prior to diagnosis. Also, infections diminished considerably as a result of IgG therapy, although this effect was considerably less for acute and chronic sinusitis. Second, the development of chronic pulmonary disease and non-infection related complications was not halted by adequate immunoglobulin therapy. A considerable number of CVID patients already had complications at

the time of diagnosis and this number of patients increased despite immunoglobulin therapy. In our cohort an association between immunological parameters and the specific complications related to CVID could be established within the B and T cell compartment.
The time to diagnosis in the group of patients with complications was significantly longer comparable to the group of patients without complications, and especially patients presenting with autoimmune phenomena are often under diagnosed. It remains important to increase awareness among doctors for the variable clinical presentations and manifestations of CVID. Specific disease related therapy would be started in an earlier stage of the disease which could affect morbidity and mortality.

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4

Outcome of gastrointestinal screening in common variable immunodeficiency and X-linked agammaglobulinemia

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Abstract

Background & Aims

Gastrointestinal (GI) symptoms and disease is common in patients with Common variable immunodeficiency (CVID) and less frequent in X-linked agammaglobulinaemia (XLA) although the exact prevalence has not well been established. The aim is to determine the prevalence and spectrum of GI disease in an outpatient cohort of patients with CVID and XLA.

Methods

A cross sectional study was performed in patients with CVID (n=61) and XLA (n=9) employing upper and lower endoscopy, biopsies, stool culture and questionnaires.

Results

Before the study 11 (18%) of the 61 CVID patients and three of the nine XLA patients had been diagnosed with gastrointestinal disease. In the current study, 25 (83%) of the 30 participating CVID and three of participating four XLA patients new endoscopic and/or histological abnormalities were detected of which 9 (30%) with therapeutic consequences including esophagitis, *Helicobacter pylori*-associated gastritis and adenoma. *Helicobacter pylori*-associated gastritis, adenomatous polyps, and nodular lymphoid hyperplasia were most frequently encountered; no malignancies were detected. Adenomatous polyps were found in two of the four patients with XLA at a relative young age. In only 44% of CVID patients symptoms could be correlated with endoscopic findings. Moreover, 40% of the asymptomatic patients were found to have gastrointestinal abnormalities, such as *Helicobacter pylori*-associated gastritis which is important in the light of the increased incidence of gastric carcinoma in CVID. The median age of CVID patients with new findings did not differ significantly from the patients without endoscopic abnormalities except for the patients with an adenoma (53 years with adenoma compared to 31 years without adenoma, $p<0.05$).

Conclusion

Gastrointestinal pathology is frequent in CVID patients regardless of symptoms. Patients with XLA seem to be at risk for colorectal adenomas at a young age. Prospective studies are needed to establish if repetitive screening is warranted.

Introduction

Common variable immunodeficiency (CVID) and X-linked agammaglobulinaemia (XLA) are primary antibody deficiency syndromes characterized by a-or hypogammaglobulinaemia and recurrent (respiratory) infections. The hypogammaglobulinaemia in CVID results from defects in B-cell differentiation, however, T cell dysregulation may also be present (1-3). The estimated prevalence of CVID is 1:10.000 to 1:100.000 (4-6). Although a hereditary pattern is not present in most CVID cases, associated gene defects have been found in individual cases (7-9). X-linked agammaglobulinaemia (XLA) is a hereditary primary antibody immunodeficiency caused by mutations in the gene for Bruton tyrosine kinase (BTK) resulting in the deficient development of B lymphocytes (10;11). XLA occurs in a frequency of about 1 in 100,000.

Complications due to the underlying immune dysregulation (3;12;13) are common in patients with CVID and include lymphoproliverative disease (14), autoimmune disease such as auto-immune cytopenias (15), (granulomatous) inflammation of organs , and an increased risk of cancer. The gastrointestinal (GI) tract is frequently affected in CVID patients. Diarrhoea is common and occurs periodically in about 20-60% of patients (3;16-20). The spectrum of GI diseases in CVID includes infections (e.g., Gardiasis, Campylobacter spp.), *Helicobacter pylori*-associated gastritis, autoimmunity (pernicious anaemia), chronic inflammatory conditions (colitis) and an increased risk of cancer, especially gastric cancer (3;18;19;21-26). Often, the inflammatory disorders mimic celiac disease, inflammatory bowel disease or pernicious anaemia although they may show significant differences in histology when compared to these disorders in immune-competent individuals (27). In contrast, GI disease is less common in patients with XLA (28), although infectious diarrhoea and GI malignancies have been reported (29;30). The exact frequency of GI pathology and the related symptomatology in patients with CVID and XLA is largely unknown. Likewise, no data are available on the role of endoscopic screening in asymptomatic patients. However, given the assumed risk and implications of GI malignancies in patients with CVID and XLA,

routine evaluation of the GI tract in all patients has been advocated by some investigators (28;31), whereas others advice screening only for symptomatic patients symptoms (32). The aim of this cross-sectional study was to determine the spectrum and frequency of gastrointestinal disease in patients with CVID and XLA, irrespective of the presence of gastrointestinal symptoms.

Methods

Patients

All adult patients diagnosed with CVID (n=61) and XLA (n=9) that were in care at the department of Internal Medicine and Infectious diseases of the University Medical Centre Utrecht were considered for enrolment in this screening. All patients had been diagnosed according to the European Society for immune deficiencies (ESID)/Pan-American Group criteria for Immunodeficiency (PAGID) (33). Approval of the Ethic committee was not necessary for this part of the study since several experts in the field have suggested endoscopic evaluation every 2-5 years for CVID and XLA patients (4;5;34;35). However, informed consent was obtained for additional extra biopsy sampling, stool culture and the questionnaire separately. Gastroscopy and colonoscopy and biopsies for histological assessment, stool culture and a questionnaire were performed in patients who had given informed consent irrespective of the presence of GI symptoms.

Evaluation of gastrointestinal history

Patients records and the results of prior endoscopies and histology of biopsies of all 61 CVID and nine XLA patients were retrospectively evaluated for previous GI disease. The time to diagnosis of CVID and XLA was defined as the time in years between the year of onset of disease-related symptoms and the year of diagnosis.

Endoscopy and histology

All endoscopies were carried out between March 2009 and April 2011 by the same gastroenterologist and the endoscopic features were registered. Targeted biopsies were taken in case of endoscopic

abnormalities and random biopsy specimens were taken of the gastric antrum and corpus, duodenum, ileum and at every 10 centimetres of the colon. For histological assessment the specimens were stained with standard hematoxylin and eosin. Additional (immunohistochemical) staining was performed when indicated. The following features were evaluated in particular: the presence of acute or chronic inflammation, villous atrophy classified according to the Marsh-Oberhuber criteria (36), intraepithelial lymphocytes, plasma cells in the lamina propria, nodular lymphoid hyperplasia, metaplasia or dysplasia and the presence of *Giardia lamblia* parasites or *Helicobacter pylori*. Gastritis caused *by Helicobacter pylori* was assessed with standard H&E staining and additional immunohistochemistry for HP was performed in case of doubt. All biopsy specimens were evaluated by two pathologists.

A gastrointestinal diagnosis was established when both endoscopic and histological findings were concordant, except for the histological evidence of microscopic colitis. If histological and endoscopic results conflicted, histological slides were revised and endoscopic reports and images were reassessed. Esophagitis was diagnosed by upper endoscopy according to the Los Angeles classification, irrespective of the histological outcome (37). Nodular lymphoid hyperplasia (NLH) was diagnosed when endoscopic examination revealed prominent nodularity and histological examination showed hyperplastic lymphoid follicles with mitotic active germinal centres and well-defined lymphocytes mantles in the mucosa or submucosa (38). Mild reactive gastropathy or minimal reflux esophagitis (Los Angeles classification grade A (37)) was not considered a relevant GI finding. Colorectal polyps were classified according to the histological diagnosis. If not available, the endoscopic description was used for classification.

Stool culture and analysis for parasites

Stool samples were cultured for *Salmonella, Shigella, Campylobacter* species and *Yersinia*. Furthermore, stool was analysed for parasites with the Triple-Faeces-test that combines multiple sampling (on three consecutive days), using a fixative on day 1 and day 3 (SAF; sodium acetate acetic acid formalin), a concentration method and an easy-to-use permanent stain (chlorazol black dye) (39).

Questionnaire

A questionnaire for GI complaints was completed irrespective of consenting to endoscopic evaluation. The questionnaire evaluated the presence and duration at the current time of dysphagia, pyrosis, unintended weight loss, GI bleeding and diarrhoea. Diarrhoea was defined as loose or watery stools more than three times a day. Diarrhoea continuing for more than 30 days was considered chronic (14).

Statistical analysis

For statistical analyses the independent samples T test, Mann–Whitney U tests and Pearson's chi square tests was used (SPSS 15.0 for Windows;IBM, Armonk, New York, USA). A p-value of 0.05 or less was considered statistically significant.

Results

Gastrointestinal history

Prior to the present study 11 of 61 adult patients with CVID (18%) had been diagnosed with a GI disorder (Table 1). This included villous atrophy in 8% (n=5), *Helicobacter pylori*-associated gastritis in 3% (n=2), non-specific ileitis or colitis in 16% (n=9). Furthermore, adenoma had been detected in three CVID patients and one CVID patient had been diagnosed with anal squamous cell carcinoma. Three of the nine XLA patients (30%) had been diagnosed with a GI disorder; two of the nine patients with XLA had been diagnosed with colitis and one of these two also with multiple colonic adenomas and carcinoma. In a third XLA case (age 41 years) a tubular adenoma was detected. In all of these patients endoscopy had been performed because of GI symptoms.

Table 2 shows the GI infections that had been established in CVID and XLA patients prior to this screening by stool culture or GI biopsy examination. Three CVID patients had been diagnosed with recurrent *Giardia Lamblia* infection and recurrent Salmonella spp. had occurred in one patient. One patient had been diagnosed with *Cytomegalovirus* associated-colitis. Six XLA patients had suffered from GI infections with *Giardia Lamblia* and *Campylobacter* (*jejuni* and *lari*) (Table 2). All patients were treated by immunoglobulins at the time of the infections.

Table 1 CVID and XLA patients with a gastrointestinal history prior to the screening

	Pt	Sex	Age at GI diagnosis (years)	Gastrointestinal disease
CVID	1	♀	14	Malabsorption due to villous atrophy duodenum
			21	Chronic ulcerative terminal ileitis-colitis causing malabsorption
	2	♀	37	Malabsorption due to lymphocytic gastroenteritis/ villous atrophy jejunum and duodenum
	3	♀	57	Esophagitis
			55	Angiodysplasia colon causing anaemia
	4	♀	56	HP associated gastritis
	5	♂	28	Chronic diarrhoea based on Giardiasis and villous atrophy
			27	HP associated gastritis
	6	♂	37	Sessile polyp colon
	7	♀	26	Diarrhoea based on villous atrophy duodenum and jejunum
			24	Diarrhoea based on chronic ulcerative ileitis-colitis
			31	Rectum carcinoma
	8	♀	52	Granulomatous colitis causing gastrointestinal abscesses and fistula
	9	♀	46	Chronic diarrhoea due to chronic non-specific colitis
	10	♂	56	Polyps colon
	11	♀	8	Chronic diarrhoea due to villous atrophy duodenum
XLA	1	♂	26	Chronic non-specific colitis
			36	HP associated gastritis
			36	Anaemia and 2 synchronous colorectal adenocarcinomas and 15-20 tubular adenomas with low-grade and high-grade dysplasia
	2	♂	13	Chronic ulcerative terminal ileitis-colitis
	3	♂	41	Adenoma colon

Pt: patient; GI: gastrointestinal; HP: Helicobacter pylori
CVID: common variable immunodeficiency, XLA: X-linked agammaglobulinaemia

Table 2 Gastrointestinal infections[1] diagnosed prior to the screening

	CVID (n=61)		XLA (n=9)
	n	(%)	n
Positive stool culture and/or biopsy	16	26	6
Salmonella spp., stool culture	2*	3	0
Campylobacter jejuni and lari, stool culture	1	2	3
Giardia lamblia, stool culture and/or biopsy	10#	16	3
Helicobacter pylori, biopsy	2	3	1
Other, stool culture and/or biopsy ¶	6	10	1

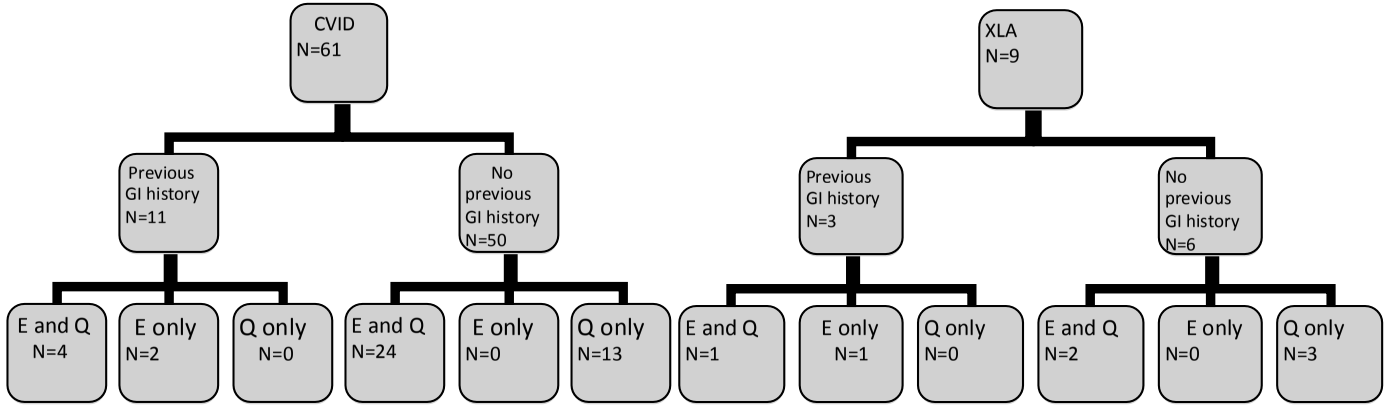
[1] Patients could have multiple gastrointestinal infections; infections are diagnosed with stool culture or gastrointestinal biopsy.
CVID: common variable immunodeficiency; XLA: X-linked agammaglobulinaemia; n= number of patients.
* 1 patient with recurrent salmonella infections
# 3 patients with recurrent Giardiasis.
¶ Other, biopsy: cytomegalovirus;
¶ Other, stool culture: Dientamoeba fragilis, Entamoeba histolytica, Blastocystis hominis, Enterobius vermicularis.

Inclusion and baseline characteristics

Thirty of the 61 CVID patients (49%) and four of the nine XLA patients consented to endoscopy, of which six and two patients had a prior GI history, respectively. Figure 1 shows the numbers of patients that participated in endoscopy and/or questionnaire.

Table 3 shows the baseline characteristics of the CVID and XLA patients who participated in endoscopic screening. The median age of the CVID patients who underwent endoscopy was 38 years (IQR 25-58 yrs.) at the time of analysis, which did not differ from the age of the patients who did not participate in the endoscopic screening (39 years (IQR 29-58 yrs.; p=0.7). The median age of the four XLA patients that underwent screening endoscopy was 28 years (IQR 21-45 yrs.) compared to 42 years (IQR 26-43 yrs.) of patients who did not participate in the endoscopic screening (p=0.8). All patients had started immunoglobulin therapy within two years after diagnosis of CVID and XLA.

Figure 1 Flowchart with inclusion of CVID and XLA patients



GI= gastrointestinal;
CVID: common variable immunodeficiency, XLA: X-linked agammaglobulinaemia
E= Endoscopy
Q= Questionnaire

Table 3 Baseline characteristics CVID and XLA patients

	CVID (n=30)	XLA (n=4)
Male[1]	11 (37)	4
Female[1]	19 (63)	na
Caucasian[1]	27 (90)	4
Age at screening*	38 (25-58)	28 (21-45)
Median age at diagnosis immunodeficiency*	25 (13-36)	2,5 (1,3-12)
Median follow up since diagnosis immunodeficiency*	7 (2-15)	20 (16-26)
Previous GI diagnosis[1]	6 (20)	2

CVID: common variable immunodeficiency; XLA: X-linked agammaglobulinaemia; GI: gastrointestinal; n= number of patients.
Other ethnicities: Middle East, Far East or mix;
* median years (interquartile range)
[1] nr of patients (%)

Screening endoscopy and histology

Endoscopy revealed (new) abnormalities in 25 (83%) of 30 CVID patients. Based on the endoscopic findings, nine of these CVID patients received medical treatment, sometimes for more than one condition (e.g. proton pump inhibitors, *Helicobacter pylori* eradication, and polypectomy. Consequent surveillance colonoscopy was appropriate in four patients because of adenoma.

Gastroscopy revealed abnormalities in eight of 30 patients (27%) and colonoscopy in 23 of 30 patients (77%). The age of patients with abnormalities on gastroscopy or colonoscopy did not differ significantly as compared to patients with no abnormalities (gastroscopy median age 45 yrs., IQR 31-59 yrs. vs. 34 years, IQR 25-59 yrs.; p=0.5; for colonoscopy median age 42 yrs., IQR 25-61 yrs. vs. 31 years, IQR 25-58 yrs.; p=0.5).

New abnormalities were detected in four of six CVID cases (67%) with a prior gastrointestinal diagnosis, i.e. colorectal low grade tubular adenoma, ileitis, nodular lymphoid hyperplasia and HP gastritis (Table 4). In only one case, the findings at endoscopy could explain the symptoms of the patient (namely diarrhoea in a patient with an ileitis). The median time that had passed since the last upper and lower endoscopy was six years (IQR 2-11 yrs.) and three years (2-6.5 yrs.), respectively.

Table 4 New endoscopic and histological findings in CVID and XLA patients after endoscopy

	CVID		XLA	
	Previous GI disease n=6 n (%)	**No previous GI disease** n=24 n (%)	**Previous GI disease** n=2 n (%)	**No previous GI disease** n=2 n (%)
No endoscopic or histological findings	2 (33)	3 (13)	0	1
Endoscopic or histological findings[1]	4 (67)	21 (87)	2	1
Esophagitis	0	4 (17)	0	0
Endoscopy		4		
Biopsy		1/4[2]		
Gastritis/ bulbitis	1 (17)	5 (21)	0	0
Endoscopy	1	5		
Biopsy	1	5		
H.pylori infection	1 (17)	2	0	0
Villous atrophy	0	1 (4)	0	0
Inflammation ileum	1 (17)	1 (4)	0	0
Endoscopy	0	0		
Biopsy	1	1		
Inflammation colon	0	5 (21)	1/2	0
Endoscopy		2/5	1	
Biopsy		4/5	1	
Polyps/adenoma	1 (17)	7 (29)	1/2	1/2
Malignancy	0	0	0	0
Nodular lymphoid hyperplasia	1 (17)	15 (63)	0	0
Endoscopy	1	15/15		
Biopsy	1	15/15		

CVID: common variable immunodeficiency, XLA: X-linked agammaglobulinaemia
n= number of patients; GI= gastrointestinal.
[1] Patients could have multiple gastrointestinal findings;
[2] Oesophageal biopsy was performed in 2 patients.

Abnormalities were identified in 21 of 24 CVID patients (88%) without a prior GI history (Table 4). The age of these 21 patients did not differ significantly from the three patients without findings at endoscopic screening (median age 32 yrs. (IQR 25-53 yrs.) vs. 26 years (IQR 20-58 yrs.), p=0.7).

Colorectal polyps were found in seven of these 21 patients, of which three could be sampled for histopathological examination revealing tubular adenoma with low grade dysplasia for which long term screening was advised. Patients with an polyp were older compared to those without (53 years (IQR 46-61 yrs.) vs. 26 years (IQR 23-32 yrs.), respectively, p= 0.006). Notably, nodular lymphoid hyperplasia was found in 16 of 30 CVID cases (53%). *Helicobacter pylori*-associated gastritis was found in three patients with CVID (10%), who were all asymptomatic. No malignancies were found. In one of the two XLA patients with prior GI history new findings were detected (microscopic colitis and a tubular adenoma with low grade dysplasia; age 50 years). In one patient with XLA (age 26 years) without a prior gastrointestinal diagnosis, hamartomatous polyps were detected. No malignancies were diagnosed.

Gastrointestinal symptoms

Of the 28 patients with CVID who participated in both endoscopy and questionnaire, 18 (64%) had experienced symptoms (mainly stomach related); however, in only eight patients (44%) could these symptoms be related to the endoscopic findings. The most frequent complaints were stomach related and diarrhoea. In 8 of the 10 asymptomatic patients endoscopic abnormalities were detected of which four (40%) appeared to be clinically relevant (*Helicobacter pylori*-associated gastritis, esophagitis, chronic non-specific colitis, and an adenoma). Notably, symptoms were less frequent in the 13 patients who filled in a questionnaire without participating in endoscopic screening (3 of 13 patients; 23%). In the majority of patients with diarrhea (71%), no satisfactory explanation could be found. The three patients with *Helicobacter pylori*-associated gastritis had no upper GI-related symptoms and in four of the five patients with unintended weight loss matching endoscopic/histological findings were detected (e.g. gastritis, villous atrophy, and ileitis/colitis) (Table 5).

In the patients with XLA, symptoms did not correlate with the endoscopic findings.

Table 5 Gastrointestinal symptoms and associated findings with endoscopy

	CVID patients without previous GI history (n=24)			**CVID patients with previous GI history (n=4)**		**XLA patients without previous GI history (n=2)**	**XLA patients with previous GI history (n=1)**
	n	%	Associated findings	n	Associated findings	n	n
No gastrointestinal symptoms	8	33	3/8*	2	2*	1	1
Gastrointestinal symptoms [1]	16	67	7/16	2	2	1	0
Dysphagia	0			0	0	0	0
Pyrosis	10	42	5/10	1	0	1	0
Stomach pain	5	21	0/5	0	0	0	0
Occasional diarrhoea[2]	2	8	1/2	1	1	0	0
Chronic diarrhoea[3]	5	21	1/5	1	1	0	0
Gastrointestinal bleeding	1	4	0/1	1	0	0	0
Unintended weight loss	5	21	4/5	0	0	0	0

CVID: common variable immunodeficiency; GI: gastrointestinal; n: number of patients;
Associated findings: number of patients with symptoms caused by GI findings found by screening endoscopy.
[1] Patients could have multiple GI symptoms;
[2] Diarrhoea: loose stools more than 3 times a day;
[3] Chronic diarrhoea: diarrhoea more than 30 days in duration
* Number of patients with GI findings without having GI symptoms.

Stool cultures and parasite analysis

The GI biopsies and random stool cultures yielded a positive culture and/or biopsy in six of the 21 CVID patients (24 %) and none of the four XLA patients (Table 6). *Giardia Lamblia* was found in both biopsy and stool analysis in a patient who reported weight loss and loose stools. In another patient with a previous GI history of chronic diarrhoea and chronic inflammation of the ileum a stool culture showed *Campylobacter jejuni*. Both patients received adequate treatment. One patient with chronic diarrhoea was found to have oxyuriasis, however she also had a previous history of chronic ulcerative ileitis and colitis.

Table 6 Gastrointestinal infections found by screening stool culture or gastrointestinal biopsy

	CVID (n=21/30)	
	n	%
Positive stool culture and/or biopsy	6	20
Salmonella, stool culture	0	
Campylobacter jejuni, stool culture	1	3
Giardia lamblia, stool culture and/or biopsy	1	3
Helicobacter pylori, biopsy	3	10
Other*, stool culture	3	10

CVID: common variable immunodeficiency; n= number of patients;
Of 21/30 patients, faeces was examined, patients can have more than one gastrointestinal infection.
* Other: Dientamoeba fragilis, Blastocystis hominis and oxyuriasis.

Discussion

The gastrointestinal tract is the largest immune organ in the body and serves as an important barrier to ingested foreign antigens. It can therefore be expected that defects in the immune system may result in considerable gastrointestinal pathology (40;41). The reported incidence of gastrointestinal disorders in patients with CVID ranges from 20% to 60% in various retrospective studies, including *Helicobacter pylori*-associated gastritis, villous atrophy, *Giardia lamblia* infections, nodular lymphoid hyperplasia and colitis (3;18;19;24-27;42;43).

In the current study, 18% of the adult patients with CVID in care at our outpatient clinic had previously been diagnosed with a gastrointestinal disorder, in line with the results from a retrospective cohort study (3), in which 21% of patients were found to have gastrointestinal disease.
The current study revealed new gastrointestinal pathology in 83% of patients with CVID, of which 9 (30%) with therapeutic consequences including esophagitis, *Helicobacter pylori*-associated gastritis and adenoma. As expected, patients with adenomas were found to be significantly older than patients without adenomas, which is in line with data from population studies (44). The prevalence of chronic diarrhoea (25%) in our cohort concurred with reports in the literature (23-60%) (34;45).
In 44% of the CVID cases, symptoms could be correlated with endoscopic findings. Moreover, 40% of the asymptomatic patients were found to have gastrointestinal abnormalities, indicating that the presence or absence of symptoms cannot be used to select patients for endoscopic screening, although numbers in this study may be too small to draw firm conclusions.
The percentage of patients with endoscopic abnormalities might be overestimated, as patients without symptoms might be less likely to consent to endoscopic screening. This is illustrated by the fact that 23% of patients who only completed a questionnaire but did not consent to endoscopic screening reported symptoms versus 64% of patients who participated in the endoscopic studies.
To determine the value of surveillance endoscopy it is important to differentiate between findings with therapeutic consequences (e.g. *Helicobacter pylori*-associated gastritis, esophagitis, villous atrophy, malabsorption, (microscopic) colitis, adenoma or malignancy) and those without (e.g. NLH).
Even when the latter category is not included, 30% (9/30) of patients with CVID had clinically relevant findings resulting in medical therapy or follow-up (e.g. *Helicobacter pylori*-associated gastritis, esophagitis grade B/C, adenomatous polyp (polypectomy), Surveillance colonoscopy was indicated in four of these patients. Additionally, three patients were treated for an infection.

The risk of cancer, such as lymphoma and gastric cancer, in patients with CVID is reported to be increased (21-23), therefore, endoscopic screening is focussed on the detection of *Helicobacter pylori*-associated gastritis associated with a further increased risk of gastric cancer (46;47) and colorectal adenoma. In the current study, *Helicobacter pylori*-associated gastritis was found in three of 30 patients with CVID (10%), who were all asymptomatic. Previous studies have reported a prevalence of HP gastritis of 8-41% in CVID patients (17;19). In the literature dyspepsia is reported in almost 50% of CVID patients, whereas gastric pathology is found in half of these cases (17). Thus, screening for *Helicobacter pylori*-associated gastritis in patients with CVID seems appropriate given the frequency of *Helicobacter pylori*-associated gastritis. In accordance, a gastric cancer surveillance protocol has recently been proposed for patients with CVID, with *Helicobacter pylori* screening by urea breath test (if available) or by upper endoscopic surveillance (31).

Colorectal polyps are generally asymptomatic however adenomas should be eradicated because of the risk of malignant progression. Polyp surveillance has shown to prevent death from colorectal cancer(48;49). In the current study, higher age was found to be a risk factor for adenomatous polyps, which is in line with data from the general population (18% in patients aged 50 years, increasing thereafter) (44). Therefore, we feel that screening for adenoma in patients with CVID can be included within the same screening programs offered to individuals from the general population.

In the current study, NLH was detected in 53% of patients with CVID, which is substantially higher than that reported in the literature (8%–18% in CVID(3;19;34;45;50) and 0.1%–1.6% in adults without primary antibody deficiency (51-53)). NLH is usually asymptomatic (54) but occasionally may cause intussusceptions leading to bowel obstruction or bleeding (54-57). In previous studies, lymphomas of the small bowel seem to occur slightly more often in adults with NLH (54). However, results should be interpreted with caution as the level of evidence is low and currently no clear guidelines exist for follow-up of these patients.

Patients with XLA present less frequently with GI pathology when

compared to other primary antibody deficiencies (42). However, chronic or recurrent diarrhoea has been reported in 23% in 201 XLA patients (11). Cases of gastrointestinal malignancies in young patients with XLA have been reported in case series (11;22;30;58-60) and the estimated risk of colorectal cancer exceeded that of the general population by 30-fold (29). Colorectal carcinoma was diagnosed in three of 52 patients (5,8%) in a Dutch study resulting in a 30-fold excess of colorectal cancer compared to the general Dutch population (29). Three of the nine XLA patients in our study had a GI history before screening. Notably, one patient was diagnosed with Crohn's disease. To our knowledge, only Washington et al(18) have previously reported a case of Crohn like disease in the small bowel of a patient with XLA. In the current study, colorectal polyps were found in two of the four patients with XLA in addition to two patients already known to have adenomatous polyps. One of these patients was diagnosed with colorectal cancer at the age of 37 years. Thus, endoscopic surveillance in patients with XLA might be recommended.

In summary, GI pathology is frequent in CVID patients regardless of (the presence or absence of) symptoms. In addition, XLA patients seem to be at risk for colorectal adenomas at a young age.
Results from the current case series of a small and relatively young cohort, do not indicate that colonoscopic screening for adenoma in patients with CVID is necessary at an earlier age. However, screening for *Helicobacter pylori* seems appropriate given the frequency and risk of *Helicobacter pylori*-associated gastritis and the potential for associated carcinoma. Larger and prospective studies with repeated endoscopies are needed to determine the frequency of endoscopic screening.

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5

Thin slice computed tomography scan reveals a high prevalence of pulmonary abnormalities in patients with Common Variable Immunodeficiency

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(Submitted)

Abstract

Background

Pulmonary disease is common in patients with common variable immunodeficiency (CVID) and involves infections as well as chronic airway disease and interstitial lung disease. However, the prevalence of pre-clinical pulmonary abnormalities in CVID patients is unknown.

Methods

The prevalence and spectrum of chronic (pre-clinical) pulmonary disease was determined in a cohort of adult CVID patients by thin slice CT scan and pulmonary function testing. CT Scans were scored for airway abnormalities and interstitial lung disease (ILD) using a scoring system.

Results

Significant pulmonary abnormalities were detected in 24 of the 47 participating patients (51%), consisting of airway disease in 30% and ILD in 34% of cases.

Significant airway or ILD pathology on thin slice CT scan could not be related to abnormalities in pulmonary function testing. In only 7 (29%) of the 24 patients with significant CT abnormalities pulmonary function test proved abnormal. The presence of significant airway disease was correlated to (recurrent) lower respiratory tract infections despite IgG therapy. Furthermore, the presence of ILD was correlated to a prolonged time (>1 year) of IgG trough levels < 8 g/L and to the presence of other CVID complications such as autoimmunity and enteropathy.

Conclusion

We have shown that airway disease and ILD are common features in CVID patients irrespective of symptoms. In order to develop a prediction model to define which patients are at risk for pulmonary complications prospective follow- up studies are necessary.

Introduction

Common Variable Immunodeficiency (CVID) is a primary antibody deficiency syndrome characterized by hypogammaglobulinaemia and recurrent respiratory tract infections caused by B cell dysfunction and in some cases additional T-cell dysregulation. CVID can be complicated by autoimmunity, inflammatory organ disease, lymphoid proliferation, gastrointestinal disease and an increased risk of malignancy (1;2). Chronic pulmonary diseases are common in CVID. Most common are asthma and chronic obstructive pulmonary disease (3), followed by structural airway disease (AD) and interstitial lung disease (ILD) (3). Structural airway disease is generally the result of recurrent and / or persistent lower respiratory tract infections causing airway wall thickening, air trapping and bronchiectasis. Bronchiectasis has been described in 4–76% of CVID patients (1;4-9) and has been associated with recurrent infections, a delayed diagnosis of CVID and inadequate treatment (5;6). Immunoglobulin replacement therapy and prophylactic antibiotics have proven to be effective in preventing pulmonary infections (10-12) and may thus indirectly prevent (the worsening of) airway disease (e.g. bronchiectasis) (13-15). However, it has been shown that chronic airway disease may still develop or progress despite adequate immunoglobulin replacement therapy (5;7) suggesting that additional factors other than infections contribute to its development (16-19). Interstitial lung disease (ILD) includes granulomatous lung disease, lymphoid interstitial pneumonia, organizing pneumonia and lymphoproliverative disorders. ILD may be caused by immune dysregulation associated with B - and T-lymphocyte dysfunction and consequent dysregulation of cytokines (interleukin-6 and TNFα) (20;21)(22). Pulmonary abnormalities such as nodules, ground glass phenomena, and reticulations can be found on CT scans. Steroids have proven to be effective in the treatment of ILD in CVID. Cyclosporine and monoclonal antibodies against TNFα are considered to be alternatives although studies are lacking (23;24). Chronic pulmonary disease in CVID patients has been associated with an increased mortality (1) and therefore early detection and monitoring

of progression will be essential to prevent progressive lung disease by additional therapeutic measures. Earlier studies support the use of an initial chest computed tomography (CT) at the time of CVID diagnosis. Recommendations for follow up CT scans range between 1-5 years to evaluate the development or progression of pulmonary disease (5;25). Alternatively, to minimize the risks of X-ray exposure in CVID patients (26) CT scanning at intervals of 4–5 years with interim annual pulmonary function testing may be more appropriate (7;27;28). However, the correlation between pulmonary function testing and findings on CT is often weak (29;30).

The purpose of this study was to describe the prevalence of thin slice CT abnormalities in CVID patients by using a standardized CT scan scoring system recently described in a paediatric CVID population (31;32) and to correlate these findings with results of pulmonary function tests and IgG trough levels.

Methods and Materials

Study Population

Between 2008 and 2012 47 of the 61 adult CVID patients in care at the department of Internal Medicine and Infectious diseases of the University Medical Centre Utrecht were screened for pulmonary disease as part of a structured follow-up by thin-slice computed tomography (CT) scan of the chest and pulmonary function testing, irrespectively of symptoms. The remaining 14 patients had recently undergone conventional CT scanning or refused for various reasons. All patients had been diagnosed according to the European Society for immune deficiencies (ESID)/Pan-American Group criteria for Immunodeficiency (PAGID) (33). Explicit approval of the Ethic committee was not necessary for this study since CT scanning is considered standard care of CVID patients (7;27;34;35).

Clinical evaluation

The medical hospital records of all patients were evaluated for clinical and laboratory data and the results of prior imaging. The time to CVID diagnosis was defined as the time (yrs.) between the year of onset of

disease-related symptoms and the year of diagnosis. A pneumonia was defined according to the SWAB/NVALT (36) or alternatively as a physician-diagnosed pneumonia. Chronic obstructive pulmonary disease (COPD) was defined according to the GOLD criteria (37). Asthma was defined according to the Ginasthma guidelines (38). Interstitial pulmonary disease was classified as granulomatous lung disease (GLILD), lymphoid interstitial pneumonia, organizing pneumonia or lymphoproliverative disorder of the lung. GLILD was confirmed with biopsy whereas the other conditions were radiological diagnoses. Other CVID-related complications were classified according to earlier reports by Chapel (1;39), I. autoimmunity (e.g. auto-immune cytopenia and organ- specific autoimmunity); II. polyclonal lymphocytic infiltration, or granulomatous disease or hepatosplenomegaly III. enteropathy; IV. malignancies.

IgG trough levels

In all patients IgG trough levels had been measured once or twice a year. The median IgG trough level over a given set of years was defined as the average IgG level per year divided by the number of years.

Thin slice CT and scoring

Thin slice CT followed a routine protocol using a 16-detector-row CT scanner (MX8000 IDT or Brilliance-16), a 64-detector-row CT scanner (Brilliance-64) or a 256-detector-row scanner (Brilliance iCT, all Philips Healthcare, Best, The Netherlands). Inspiratory scans were acquired in a caudocranial direction using 130 mAs and 100-120 kVp (depending on body weight) and expiratory scans in a caudocranial direction using 20 mAs and 80-100 kVp (depending on body weight). Images were reconstructed with a slice thickness of 1-mm (16-detector-row and 256-detector-row scanners) or 0.9-mm (64-detector-row scanner) and an increment of 0.7-mm. The presence of airway disease (AD) and interstitial lung disease (ILD) was scored in each lobe by an independent observer (P. de J.) according to a previous published scoring system (31;32;40). The CVID airway disease score is a composite of bronchiectasis, airway wall thickening, mucus plugging, tree-in bud and air-trapping. CVID interstitial disease score is a composite of

opacities, ground glass, septa thickening and lung nodules. Signs of AD were scored by assessing the presence of bronchiectasis, airway wall thickening, mucus plugging, tree-in-bud and air-trapping. The ILD score was based on the presence of opacities, ground glass, septa thickening and lung nodules. As previously described an ILD score > 5 and an AD score > 7 was defined as clinically significant (31;32).

Pulmonary function testing

All pulmonary function tests were performed using standardised equipment according to the current European Respiratory Society (ERS)/American Thoracic society guidelines (41-43) and consisted of spirometry and body plethysmography (forced expiratory volume in one second (FEV1), forced vital capacity (FVC), FEV1/FVC ratio, vital capacity (VC), residual volume (RV), total lung capacity (TLC) and transfer coefficient for carbon monoxide (Kco). All Spiro metric values were obtained after inhalation of a bronchodilator. All values are presented as percentages of the predicted values (44). The lower limits of normal were calculated using the reference equations of the European Community of Coal and Steel (ECCS) (44). Airway obstruction was diagnosed when the FEV1/FVC was <80% of the predicted value. Diffusion capacity was considered abnormal when measured <75% of the predicted value (44;45). Each test was evaluated by an independent observer (J.W.L).

Data Analysis

Data were analysed using SPSS 20.0 (SPSS Inc.; Chicago, IL). Statistical analyses were performed using Mann–Whitney U tests in case of continuous variables and Chi square tests for categorical variables. Statistical significance was determined at $p < 0.05$.

Results

Between 2008 and 2012, 47 patients in care were evaluated by thin slice CT scan (6 patients without expiration and 41 patients with expiration). The baseline characteristics of the patients are shown in Table 1. The median age was 37 years (IQR 29-54 yrs.) at the time of the CT scan. The

Table 1 characteristics of CVID study population.

Nr. of patients, n=47	**yrs (IQR)**	
Median age at time of scan	37 (29-54)	
Median age at diagnosis immunodeficiency	27 (16-38)	
Median time to diagnosis	9,5 (5-16)	
Median follow up since diagnosis immunodeficiency	9,5 (6-15)	
	g/L (IQR)	
Median IgG trough level/yr ,	8,9 (7,7-9,9)	
Median IgG level at diagnosis,	3,9 (2,3-4,8)	
	Nr. of Patients	%
Male	16	34%
Ethnicity, caucasian	42	89%
Smoking	13	28%
Previous (Hr)CT	10/47	21%
Normal	4	
Airway disease	5	
ILD	3	
Respiratory tract Infections		
pneumonia before start of therapy	13	28%
pneumonia after start of therapy	7	15%
Established pulmonary disease	16	34%
Asthma/COPD	9	19%
Interstitial lung disease	3	6%
Bronchiectasis	4	9%
CVID related complications*		
None	24	51%
Auto Immune disorders	8	17%
Lymphoproliferative disorders	12	26%
Enteropathy	10	21%
malignancy	4	9%
Number of complications per patient*		
0	24	51%
1	14	30%
2	7	15%
3	2	4%

Baseline characteristics 47 CVID patients who underwent thin slice CT scan (six patients without expiration and 41 patients with expiration).
CVID: Common Variable Immunodeficiency; (Hr) CT scan: (High resolution) Computed Tomography scan; IQR: inter quartile range; yrs: years
ILD: Interstitial lung disease; COPD: Chronic obstructive pulmonary disease.
*according to Chapel classification(1)

median time to CVID diagnosis had been 9.5 years (IQR 5-16 yrs.). Half of the patients (23/47, 49%) had been diagnosed with one or more CVID related complications such as auto immune- or lymphoproliverative conditions. One of the patients died from a non-pulmonary cause. Sixteen of the 47 patients (34%) had already been diagnosed with a symptomatic pulmonary condition of which nine (19%) with asthma, three with ILD (6%) compared to 43% of all CVID patients in care. Three patients (6%) had already been known with bronchiectasis. In 10 of the 47 patients a conventional CT scan was available ranging between 3.5-14.5 years ago, however the CT scoring system from the current study could not be applied to earlier conventional CT scans and therefore progress could not be assessed.

In 45 of 47 CVID (96%) patients one or more CT abnormalities were scored (Table 2). In 40 of 47 (85%) patients the airway disease (AD) score was ≥1, which was most commonly based on air wall thickening (35 patients, 75%) and/or the presence of bronchiectasis. The score for bronchiectasis varied from 2 to 32 (30 patients, 64%). In 34 patients (72%) the ILD score was ≥1, which was most commonly based on the presence of nodules (26 patients, 55 %).

An AD score >7 or an ILD score >5 was considered indicative of significant disease based on an earlier report using this scoring system (31;32). In 24 patients (51%) CT abnormalities were considered significant for AD, ILD or both. In 14 of the 47 patients (30%) the AD score was significant and the ILD score in 16 of 47 patients (34%).

Table 3 and 4 shows the clinical differences between patients with and without significant airway disease or ILD, respectively. No correlation was found between current age, the age at diagnosis or the time to diagnosis on the one hand and the presence of significant airway disease or/ILD (AD score>7; ILD score>5) on the other hand. Patients with an AD score >7 had suffered more frequently from (recurrent) pneumonia when compared to patients with an AD score ≤ 7 (p=0.009). Notably, bronchiectasis was also detected in patients who had never been diagnosed with pneumonia (17 of the 30 patients with bronchiectasis, 57%).

Table 2 Results of thin slice CT scan and pulmonary function test in 47 CVID patients

Nr. of patients, n=47	**Nr. of patients**	
Thin slice CT with expiration	41	87%
Thin slice CT without expiration	6	13%
Thin slice CT		
Any abnormality	45/47	96%
Airway disease	40	85%
Bronchiectasis	30	64%
Newly diagnosed	27	
Severity Moderate-severe	2*	
mild	28	
Airway wall thickening	35	75%
Combined mucus score #	15	35%
Airtrapping	21	45%
Airway disease score>7	14	30%
ILD	34	72%
Opacities	16	34%
Noduli	26	55%
Ground glass	16	34%
Septa thickening	9	19%
ILD score >5	16	34%
Airway disease score>7 and/or ILD score >5	24	51%
Pulmonary function test	44/47	94%
Any abnormality	10	23%
Obstructive	4	
Restrictive	3	
Mix obstructive and restrictive	0	
Diffusion abnormality	3	
Test incomplete	2	

CVID: Common Variable Immunodeficiency; ILD: interstitial lung disease; CT: Computed Tomography scan; n= number of patients.
combined mucus score: sum of mucus plugging score and tree-in-bud scores.
* patients known with bronchiectasis prior to the study.

Table 3 Clinical differences between CVID patients with and without significant airway pathology

	Number of patients with AD score ≤7 (n=33)	**Number of patients with AD score >7** (n=14)	**p value**
Nr. of patients, n=47			
Male #	9 (27%)	7 (50%)	0.1
Ethnicity, caucasian/total #	29 (88%)	13 (93%)	0.7
Median age at time of scan ¶	37 (30-52)	37 (25-61)	0.9
Median time to diagnosis ¶	10 (5-17)	9 (5-12)	0.5
Median follow up since diagnosis immunodeficiency ¶	10 (6-15)	7 (3-19)	0.5
Median IgG at diagnosis, g/L (range)	3,8 (2,7-4,7)	4,3 (0,6-5)	0.7
Median IgG trough in the last 10 years, g/L (range)	8,9 (8,2-9,9)	8,8 (7,4-9,7)	0.4
Smoking #	8 (24%)	5 (36%)	0.3
Respiratory tract Infections #			
pneumonia before Ig therapy	8 (24%)	5 (36%)	0.4
pneumonia since Ig therapy	2 (6%)	5 (36%)	**0.009**
Patients with CVID related complications* #			
None	16 (49%)	8 (57%)	0.5
Auto Immune disorders [1]	7 (21%)	2 (14%)	0.7
Lymphoproliferative disorders [2]	9 (27%)	5 (36%)	0.8
Enteropathy	6 (18%)	4 (29%)	0.4
malignancy	4 (12%)	0	0.1
Nr. of patients, n=44			
Pulmonary functiontest #	31 (94%)	13 (93%)	
Any abnormality	6 (19%)	4 (29%)	0.5
Obstructive	1 (3%)	3 (11%)	**0.04**
Restrictive	3 (9%)	0	0.5
Diffusion abnormality	2 (6%)	1 (3%)	ns

This table shows the clinical differences between patients with and without significant airway disease in CT scoring.
CVID: Common Variable Immunodeficiency disorders; AD: airway disease;
number of patients (%)
¶ In years (interquartile range)
*according to Chapel classification.(1)
[1] auto-immune: cytopenia and organ- specific autoimmunity
[2] polyclonal lymphocytic infiltration (defined as unexplained persistent lymphadenopathy, granulomatous disease or hepatosplenomegaly (ultrasound proven).

Table 4 Clinical differences between CVID patients with and without significant interstitial lung pathology

	Number of patients with ILD score ≤5 (n=31)	**Number of patients with ILD score >5** (n=16)	**p value**
Nr. of patients, n=47			
Male #	11 (36%)	5 (31%)	0.8
Ethnicity, caucasian/total #	28 (90%)	14 (88%)	0.5
Median age at time of scan ¶	34 (26-49)	42 (31-62)	0.2
Median time to diagnosis ¶	9,5 (5-16)	9 (5-18)	0.9
Median follow up since diagnosis immunodeficiency ¶	9 (5-16)	9 (4-17)	0.9
Median IgG at diagnosis, g/L (range)	4,5 (2,5-5,3)	3,4 (1,8-4,1)	0.1
Median IgG trough in the last 10 years, g/L (range)	9,1 (7,7-10)	8,9 (7,6-9,2)	0.2
Smoking #	10 (32%)	3 (19%)	0.4
Respiratory tract Infections #			
pneumonia before Ig therapy	9 (29%)	4 (25%)	0.7
pneumonia since Ig therapy	3 (10%)	4 (25%)	0.1
Patients with CVID related complications* #			
None	21 (68%)	3 (19%)	**0.001**
Auto Immune disorders [1]	2 (7%)	6 (38%)	**0.007**
Lymphoproliferative disorders [2]	6 (19%)	6 (38%)	0.2
Enteropathy	4 (13%)	6 (38%)	0.05
malignancy	1 (3%)	2 (13%)	0.2
Nr. of patients, n=44			
Pulmonary functiontest #	29 (94%)	15 (94%)	0.9
Any abnormality	6 (21%)	4 (27%)	0.7
Obstructive	3 (11%)	1 (7%)	0.7
Restrictive	2 (7%)	1 (7%)	ns
Diffusion abnormality	1 (4%)	2 (14%)	0.6

This table shows the clinical differences between patients with and without significant ILD in CT scoring.
CVID: Common Variable Immunodeficiency disorders; ILD: interstitial lung disease;
number of patients (%)
¶ In years (interquartile range)
*according to Chapel classification.(1)
[1] auto-immune: cytopenia and organ- specific autoimmunity
[2] polyclonal lymphocytic infiltration (defined as unexplained persistent lymphadenopathy, granulomatous disease or hepatosplenomegaly (ultrasound proven).

In patients with an ILD score >5 autoimmune disease was more frequent when compared to patients with an ILD score ≤ 5 (6/16 (38%) vs. 2/31 (6%) patients, p=0.007). The majority of patients without any CVID-related complications had an ILD score <5 (21 (68%) vs. 3 (19%), p=0.001).

Pulmonary function testing proved abnormal in 10 of the 44 tested patients (Table 2).

In only 7 (29%) of the 24 patients with significant CT abnormalities pulmonary function testing proved abnormal indicating the poor correlation of pulmonary function testing and findings on the CT scan.

The distribution of IgG levels at the time of CVID diagnosis and the median IgG trough levels during therapy in the last ten years did not differ between patients with or without any significant airway disease or ILD. However we found that the presence of ILD was correlated to a prolonged time (> 1 year) of IgG trough levels < 8 g/L (Figure 1).

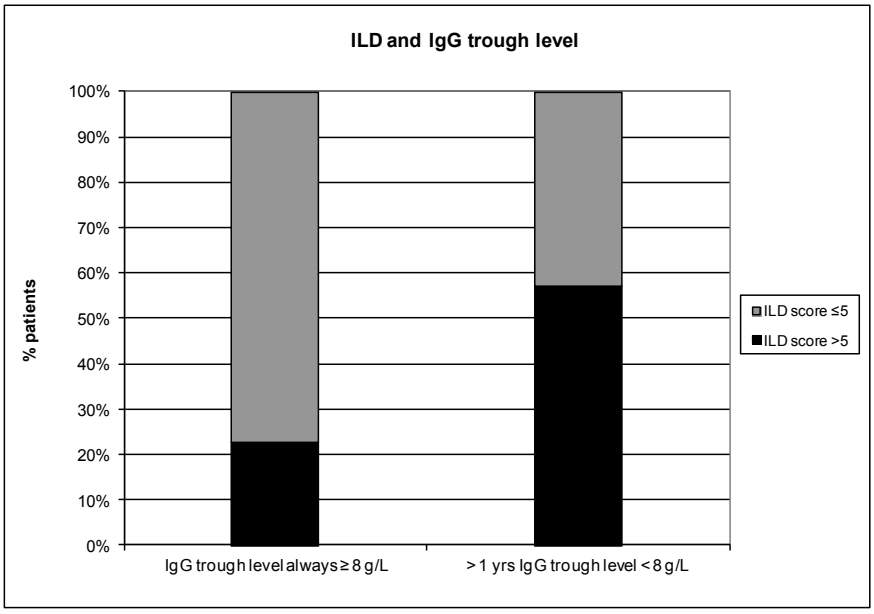

Figure 1 Correlation between the presence of ILD and the IgG trough level
This figure shows de percentage of patients with and without prolonged time of inadequate IgG trough levels, with and without significant ILD (ILD score>5). The median IgG trough level over a given set of years was defined as the average IgG level per year divided by the number of years. ILD: interstitial lung disease.

In patients with any AD abnormalities the majority (67%) had IgG trough levels that were considered adequate (>8 g/L).

Discussion

Chronic pulmonary disease is a common complication in CVID patients and may develop despite adequate immunoglobulin substitution therapy (5-7;9;46). The current cross sectional study reports on a relatively high incidence of pulmonary abnormalities using thin slice CT scanning. Subtle pre-clinical pulmonary abnormalities were detected in virtually all participating CVID patients by thin slice CT scan (AD or ILD scores ≥ 1 in 96% and 51% of patients, respectively). However, significant airway disease (AD score >7) or ILD (ILD score >5) was present in 30% and 34% of patients, respectively. The rates might even be an underestimation since the percentage of patients with established pulmonary disease that participated in the study was lower (34%) when compared to the entire group of 61 CVID patients in care (43%). When compared to children these rates are considerably higher. A recent publication that used the same scoring system in CVID children in our centre reported significant airway abnormalities in 20% and ILD in 14% of cases (31;32).

The correlation between CT scores and results of pulmonary function testing in our study was poor, indicating that pulmonary function testing is a poor predictor of early pulmonary abnormalities and that thin slice CT is a more sensitive way to detect early pulmonary disease. Bronchiectasis and airway disease have been associated with (recurrent) lower respiratory tract infections (47;48) and immunoglobulin replacement therapy (with adequate IgG trough levels) and prophylactic antibiotics have proven to be effective in preventing pulmonary infections (10-12). In our study the presence of significant airway disease (AD score >7) was indeed associated with the occurrence of pneumonia occurring after initiation of IgG therapy. However, in our analysis 17 of the 30 patients with bronchiectasis had never been diagnosed with pneumonia, although infections might have occurred subclinical. Three previous reports also demonstrated

(silent) progression of airway disease despite adequate therapy and less pulmonary infections (7;49;50). It has been proposed that IgG trough levels > 9 g/L should be attained in patients with bronchiectasis, however, evidence supporting this proposition is scarce.

Other factors such as age, duration of disease, time to the diagnosis of CVID or IgG trough level could not be identified as discriminating factors to identify patients at risk for the development of structural airway disease.

Interestingly, we did find that patients with IgG trough levels < 8 g/L for more than one year were more likely to have significant ILD abnormalities on CT scan. It is known that the therapeutic benefit of immunoglobulin therapy is not limited to antibody replacement but has also shown to be beneficial in autoimmune disease (51). However, the doses used in those treatments are usually much higher than those used for replacement therapy and the mechanism of its immunomodulatory and anti-inflammatory effects remains unclear (51). It is known, however, that recurrent infections – which are clearly related to insufficient IgG trough levels in CVID- can evoke exacerbations of inflammatory diseases (52).

Concurrent with earlier reports, ILD was associated with the presence of auto immune disease and enteropathy in our study (29;32;53). Reversely, of the patients with an ILD score ≤5 the majority had no CVID related complications.

Given the high prevalence of chronic pulmonary disease in CVID patients that is associated with mortality (1), early detection and monitoring seems essential. Repetitive CT scanning and CT scoring systems are reliable and reproducible tools to monitor progression of pulmonary abnormalities and to identify patients at risk. However, no consensus has yet been reached about the frequency of CT scanning. In the current practice the current time interval for CT evaluation ranges from 1 to 5 years (5;25). Alternatively, annual pulmonary function testing has been suggested (27;28), however, in our and other studies abnormalities on the CT scan were not clearly related to abnormal pulmonary function testing (30). Based on our and others experience we would suggest biannual CT screening of CVID patients with (I) active

autoimmune disease or other non-infectious inflammatory disease, (II) patients with on-going frequent lower respiratory tract infections and (III) patients with a significant AD (>7) or ILD score (>5). For other CVID patients CT screening once per five years might be sufficient.

Our study has several limitations. First, the number of patients was too small to develop a reliable prediction model to define which patients are at risk to develop pulmonary complications. Second, it is a cross-sectional study and a prospective follow-up study will be necessary to establish potential progression and its relation to risk factors. Third, we could not establish when the CT abnormalities had evolved (before or after the initiation of immunoglobulin therapy) and neither progression could be evaluated due to the low number of patients with prior thin slice CT scanning.

In conclusion, our study shows a relatively high frequency of significant pulmonary CT abnormalities in CVID patients despite IgG therapy and despite normal pulmonary function testing. Airway disease is correlated to pulmonary infections, but can also develop sub clinically. ILD is correlated to the presence of autoimmune disease and a prolonged time of IgG trough levels below 8 g/L. Prospective follow- up studies will be necessary to ascertain the optimal follow up frequency of these abnormalities, and for evaluation of therapeutic actions aimed at preventing the progression of pulmonary morbidity in CVID patients.

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Correlation of immunological parameters with pulmonary and gastrointestinal pathology in Common Variable immunodeficiency

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(Submitted)

Abstract

Background

Common variable immunodeficiency (CVID) is a common and heterogeneous primary antibody deficiency. CVID can be complicated by autoimmunity, malignancy and inflammatory organ disease such as chronic pulmonary disease (airway and interstitial lung disease) and gastrointestinal disease (e.g., inflammatory bowel disease, villous atrophy) associated with excess morbidity and early mortality.

Methods

To identify immunological phenotypes that might predict patients at risk, we analysed B and T lymphocyte subsets in a cohort of CVID patients with defined gastrointestinal- and pulmonary pathology. Thin slice Computed Tomography (CT) of the lung and gastrointestinal endoscopies were performed as part of the clinical follow up.

Results

In patients with signs of interstitial lung disease (ILD) on thin slice CT the median absolute numbers of CD4+ T cells, naïve CD4+ T cells were markedly reduced, as well as naïve CD8+ T cells and memory B cells. Airway disease (AD) could not be correlated to specific T- and B cell phenotypes, nor could gastrointestinal pathology. Patients with nodular hyperplasia of the small bowel however, had lower IgA levels than patients without this disease.

Conclusion

In CVID patients with signs of ILD the absolute number of the total CD4+ T cells count and the naïve CD4+ T cells are candidate immunological markers to identify patients at risk.

Introduction

Common variable immunodeficiency (CVID) is a primary antibody deficiency characterized by recurrent (respiratory) infections caused by hypogammaglobulinaemia that results from defects in B-cell differentiation. In addition, T cell abnormalities have been demonstrated in CVID patients such as a reduced thymic output (1;2), reduced T cell proliferation in response to mitogens and antigens (3), reduced CD40L expression in activated T cells (4) and increased T cell apoptosis (5). Non–infectious complications due to immune dysregulation are common in CVID patients and involve lymphoproliferative disease, autoimmunity and malignancy (6;7). Despite immunoglobulin replacement therapy, which has reduced infections and improved survival rates of CVID patients, mortality is still high. Post-diagnosis survival was estimated as 65% for the first 6.5 years and 45% after 14 years which is mainly caused by progressive end-organ disease, mainly in lungs and gut (8;9). Attempts have been made to classify the heterogeneous CVID population into subgroups by clinical complications (6;7) the analysis of B- (10-12) and T cell compartments by flowcytometry (1;13-15).

Clinically, patients can be divided in the following distinct phenotypes: (I) infections only, (II) autoimmunity, (III) polyclonal lymphocytic infiltration, (IV) enteropathy and (V) malignancies. In the classifications of the CVID population according to B cell phenotype (10;12;16) it has been shown that patients with more severe complications had lower numbers of switched memory B cells (10;12;14;17;18). A reduction in memory B cells has been associated with granulomatous-, autoimmune disease and splenomegaly (10;19). In view of the T cell abnormalities, T cell classification schemes were proposed (1;14;15;20). Overall the reduction of CD4 naive T cells was most consistent associated with CVID related complications such as autoimmunity and lymphoproliferation (14;15), splenomegaly and granulomatous disease (1). Although 32-50% of CVID patients remains free of complications (6;8;21), a substantial number of patients will develop one or more complication.

Chronic pulmonary disease is a common problem in CVID patients and can involve asthma and chronic obstructive pulmonary disease (COPD) but also CVID-related disease such as structural airway disease

(AD) and interstitial lung disease (ILD) (22). Structural airway disease is generally caused by recurrent lower respiratory tract infections that may lead to airway wall thickening, air trapping and bronchiectasis. The development of airway disease has been shown to progress despite adequate immunoglobulin replacement in some patients and even in the absence of clinically recognized infections (23;24). Interstitial lung disease (ILD) includes granulomatous lung disease, lymphoid interstitial pneumonia, organizing pneumonia and other lymphoproliverative disorders of the lungs. ILD may be caused by the additional T-lymphocyte dysfunction which is present in some CVID patients (25-27). In a recent study we detected signs of airway disease by thin slice Computed Tomography (CT) in 30% and ILD in 34% of CVID cases (Maarschalk et al, submitted for publication).

Gastrointestinal disorders are also frequent in CVID (28;29), these include pernicious anaemia, chronic inflammatory conditions such as colitis or ileitis and an increased risk of cancer (30-32). In a recent study we detected endoscopic and/or histological abnormalities in 83% of CVID patients (n=30) regardless of symptoms, and in nine of these patients the results prompted medical treatment (33). Both chronic pulmonary and gastrointestinal disease have been associated with excess morbidity and early mortality in CVID patients (6;8;23). Therefore, early detection and monitoring of progression of such conditions is essential. In the current study, we set out to identify patients at risk for gastrointestinal- and pulmonary complications by analysing peripheral blood B- and T lymphocyte subsets in a cohort of CVID patients with well-defined gastrointestinal- and pulmonary pathology and comparing the results to CVID patients without these complications.

Methods

Study Population

We included adult CVID patients in care at the department of Internal Medicine and Infectious diseases of the University Medical Centre Utrecht who had been previously screened between 2008-2012 by

chest thin slice computed tomography (CT) and/or gastrointestinal endoscopic evaluation. All CVID patients had been diagnosed according to the European Society for immune deficiencies (ESID)/Pan-American Group criteria for Immunodeficiency (PAGID) (34) and were treated with immunoglobulin replacement therapy. Explicit approval of the Ethic committee of the University Medical Centre of Utrecht was not necessary for the monitoring of immunoglobulin titres, and performing of T- and B cell phenotyping, CT scanning and endoscopy as these are considered standard care for CVID patients (23;35-39). However, informed consent was obtained for additional biopsy sampling during endoscopy.

Clinical evaluation

Thin slice CT scans had been performed and scored according to a scoring system as previously described (40;41). The presence of airway disease (AD) and interstitial lung disease (ILD) was scored per lobe on the thin slice CT scan by an independent observer. The airway disease (AD) score was based on the presence of bronchiectasis, airway wall thickening, mucus plugging, tree-in-bud phenomenon and air-trapping. The interstitial lung disease (ILD) score was based on the presence of opacities, ground glass, septa thickening and lung nodules. An ILD score > 5 and an AD score > 7 has been defined as clinically significant (as of now indicated as ILD or AD) (40;41). Endoscopy consisted of both gastroscopy and colonoscopy of which the results have previous been published (33). CVID-related complications were classified according to earlier reports by Chapel (6;7): I.autoimmunity (e.g. auto-immune cytopenia and organ- specific autoimmunity); II. polyclonal lymphocytic infiltration, granulomatous disease or hepatosplenomegaly; III. enteropathy; IV. malignancies.

Laboratory data

T- and B cell phenotyping was performed in patients between 2007 and 2011 in clinically stable patients. The T- and B cell (sub)populations were analyzed by four-color flow cytometry using whole blood and antibodies to CD3, CD45, CD27, CD4, CD8, HLA-DR, CD38, CD45RA

and CD19, CD27, CD38, CD10, IgM, IgG, IgA and IgD, respectively, as described previously(42). Within the CD19+ B cell compartment, the following populations were distinguished: IgD+CD10+CD38++ recent bone-marrow emigrants (RBE), IgM+IgD+CD27-CD10- naive B cells, non-Ig class-switched IgM+IgD+CD27+ memory B cells, IgG+CD27+ and IgA+CD27+ memory B cells. CD3+ T cells were divided into CD4+ and CD8+ subsets, and subsequently into CD38+HLA-DR+ activated T cells, CD45RA+CD27+ naïve, and non-naïve CD45RA+CD27-, CD45RA-CD27+ or CD45RA-CD27- T cells. IgG trough levels were measured once or twice a year in clinically stable patients during follow up, and more frequent in patients who were diagnosed with disease-related complications. In our current daily practice we aim to reach IgG through levels of at least 8 g/L (43).

Data Analysis

Data were analysed using SPSS 20.0 (SPSS Inc.; Chicago, IL). Continuous, non-normally distributed variable were analysed by using the Mann–Whitney U tests. Chi square tests were performed for categorical variables. Statistical significance was determined at $p < 0.05$. An analysis of the sensitivity, specificity and positive predictive value of the specific immunological markers for ILD was performed. For this analysis we used an ILD score >5 as golden standard. As the choice of a single cut-off value is arbitrary, we also calculated the c-statistic to give an overall measure of the discriminative power of the variables.

Results

A thin slice CT scan had been performed in 47 patients and endoscopic screening in 30 of the 61 patients in care, and the baseline characteristics are summarized in table 1. Significant airway abnormalities (AD) were detected in 14/47 patients (30%) and ILD in 16/47 patients (34 %) (table 1). In patients with an ILD score >5, all CVID related complications were observed more frequently when compared to patients with an ILD score≤5 (81% versus 32%). Confirming earlier studies (41;44) auto immune disease was more frequent in patients with signs of ILD when compared to patients without ILD (38% vs. 7%, p=0.007). Screening

endoscopy had revealed abnormalities in the vast majority of CVID patients (25/30, 83%) (33), which were clinically relevant in 9 patients (36%). Nodular lymphoid hyperplasia (NLH) was most prevalent (53%), followed by polyps (27%), (microscopic) colitis or ileitis (27%) and Helicobacter Pylori gastritis (10%). (33) (table 1).

Table 1 Baseline characteristics of CVID patients

	GI screening*	**Pulmonary screening***
	n=30	n= 47
Female[1]	19 (63%)	31 (66%)
Caucasian[1]	27 (90%)	42 (89%)
Median age at screening[2]	38 (25-58)	37 (29-54)
Median age at diagnosis immunodeficiency[2]	25 (13-36)	27 (16-38)
Median time to diagnosis of immunodeficiency[2]	11 (5-17)	9,5 (5-16)
Median follow up since diagnosis immunodeficiency[2]	7 (2-15)	9,5 (6-15)
GI abnormality at screening[1]	25 (83%)	
Nodular lymphoid hyperplasia	16 (53%)	
ileitis/colitis	9 (30%)	
Polyps	8 (27%)	
Pulmonary abnormality at screening[1]		24 (51%)
Airway score >7		14 (30%)
ILD score >5		16 (34%)

CVID: Common variable immunodeficiency disorders;GI: gastrointestinal;
ILD: interstitial lung disease
*GI screening comprises of patients who were screened with gastroscopy and colonoscopy
*Pulmonary screening comprises of patients who were screened with thin slice CT scan and pulmonary function tests
Other ethnicities: Middle East, Far East or mix;
[1] Number of patients (%)
[2] In years (interquartile range)

Pulmonary pathology in relation to clinical and immunological characteristics (table 2)

Patients with an ILD score >5 showed significantly different T- and B cell profiles when compared to patients with an ILD score ≤ 5, (table 2 and figure 1). The median absolute number of CD4+ T cells was significantly lower in patients with an ILD score > 5 when compared

Table 2 Clinical and immunological differences between CVID patients with and without interstitial lung disease

n=47	ILD≤5		ILD>5		p
	n=31	IQR	n=16	IQR	
Age at diagnosis, yrs	24	(11-38)	30	(21-43)	0.2
Time to diagnosis, yrs	9.5	(5-16)	9	(5-18)	0.9
Age at time of CT scan, yrs	34	(26-49)	42	(31-62)	0.2
IgG trough level during Ig replacement therapy during the last ten years, g/l	9.1	(7.7-10)	8.9	(7.6-9.2)	0.2
CVID related complications, nr of patients (%) ¶ §					
none	21	(68%)	3	(19%)	**0.001**
Auto-immune disorders[1]	2	(6%)	6	(38%)	**0.007**
Lymphoproliverative disorders[2]	6	(19%)	6	(38%)	0.2
Enteropathy	4	(13%)	6	(38%)	0.05
Malignancy	1	(3%)	2	(13%)	0.2
NK cells*	195	(110-288)	125	(96-228)	0.1
CD3+ T lymphocytes*	1589	(1329-2002)	969	(738-1856)	**0.03**
CD4 T+ lymphocytes*	989	(611-1169)	601	(344-754)	**0.03**
Activated CD4+ T lymphocytes*	9	(3-18)	12	(8-21)	0.3
Naive CD4+ T lymphocytes*	434	(290-654)	78	(22-220)	**0.004**
Effector/memory CD4+ T lymphocytes*	529	(468-621)	383	(115-523)	0.01
CD8+ T lymphocytes*	557	(312-847)	402	(229-620)	0.1
Activated CD8+ T lymphocytes*	13	(3-18)	16	(8-24)	0.8
Naive CD8+T lymphocytes*	271	(162-504)	99	(51-237)	**0.03**
Effector/memory CD8+ T lymphocytes*	297	(210-439)	185	(118-261)	0.1
CD4/8 ratio	1.6	(1.1-2.1)	1.5	(1.1-2.4)	0.7
CD19+ B lymphocytes*	266	(184-406)	132	(74-305)	0.05
Naive B lymphocytes*	162	(102-276)	78	(45-146)	**0.03**
Recent Bone Marrow Emigrants B lymphocytes*	19	(5-43)	14	(8-28)	0.2

Table 2 Continued

n=47	ILD≤5		ILD>5		p
	n=31		n=16		
IgM memory B lymphocytes*	43	(15-72)	14	(1.5-57)	0.8
IgG memory B lymphocytes*	7	(4-21)	1.5	(0.1-10)	**0.04**
IgA memory B lymphocytes*	4	(2-12)	2	(0.3-6)	0.1
Total class switched memory B lymphocytes*	11	(8-30)	6	(0.4-14)	**0.03**
Switched memory B cells≤ 2% of total B lymphocytes #	3	(14%)	5	(36%)	0,13

CVID: Common variable immunodeficiency disorders; ILD: interstitial lung disease, ILD score >5
NK cells: Natural Killer cells, n= number of patients.
Yrs: years; IQR: interquartile range; g/L: gram/liter
§ Patients could have multiple complications
*Median absolute counts per mm³ (IQR)
¶ according to Chapel classification (6,7)
# according to EUROclass trial (10).
[1] auto-immune: cytopenia and organ- specific autoimmunity
[2] polyclonal lymphocytic infiltration (defined as unexplained persistent lymphadenopathy. granulomatous disease or hepatosplenomegaly
Reference values, (all in absolute numbers): from van Gent et al, Clinical Immunology (2009) 133, 95-107
CD3+ T lymphocytes (100–400); CD4 T+ lymphocytes (400–1300); CD8+ T lymphocytes(200–700); CD4/CD8 ratio (1.1–3.2);
Activated CD4+ T lymphocytes (2.5-8.5); Naive CD4+ T lymphocytes (240-790); Effector/memory CD4+ T lymphocytes (150-500);
Activated CD8+ T lymphocytes (4-19);Naive CD8+T lymphocytes (220-400); Effector/memory CD8+ T lymphocytes (50-190);
CD19+ B lymphocytes (100-400); Naief B cells (72-257); Recent Bone Marrow Emigrants B cells (6-41);
IgM memory B cells (10-39); IgG memory B cells (2-51); IgA memory Bcells (1-20).

CD19+ B cell compartment: IgD+CD10+CD38++ recent bone-marrow emigrants (RBE), IgM+IgD+CD27-CD10- naive B cells,
non-Ig class-switched IgM+IgD+CD27+ memory B cells, IgG+CD27+ and IgA+CD27+ memory B cells.
CD3+ T cells were divided into CD4+ and CD8+ subsets, and subsequently into CD38+HLA-DR+ activated T cells, CD45RA+CD27+ naïve,
and non-naïve CD45RA+CD27-, CD45RA-CD27+ or CD45RA-CD27- T cells.

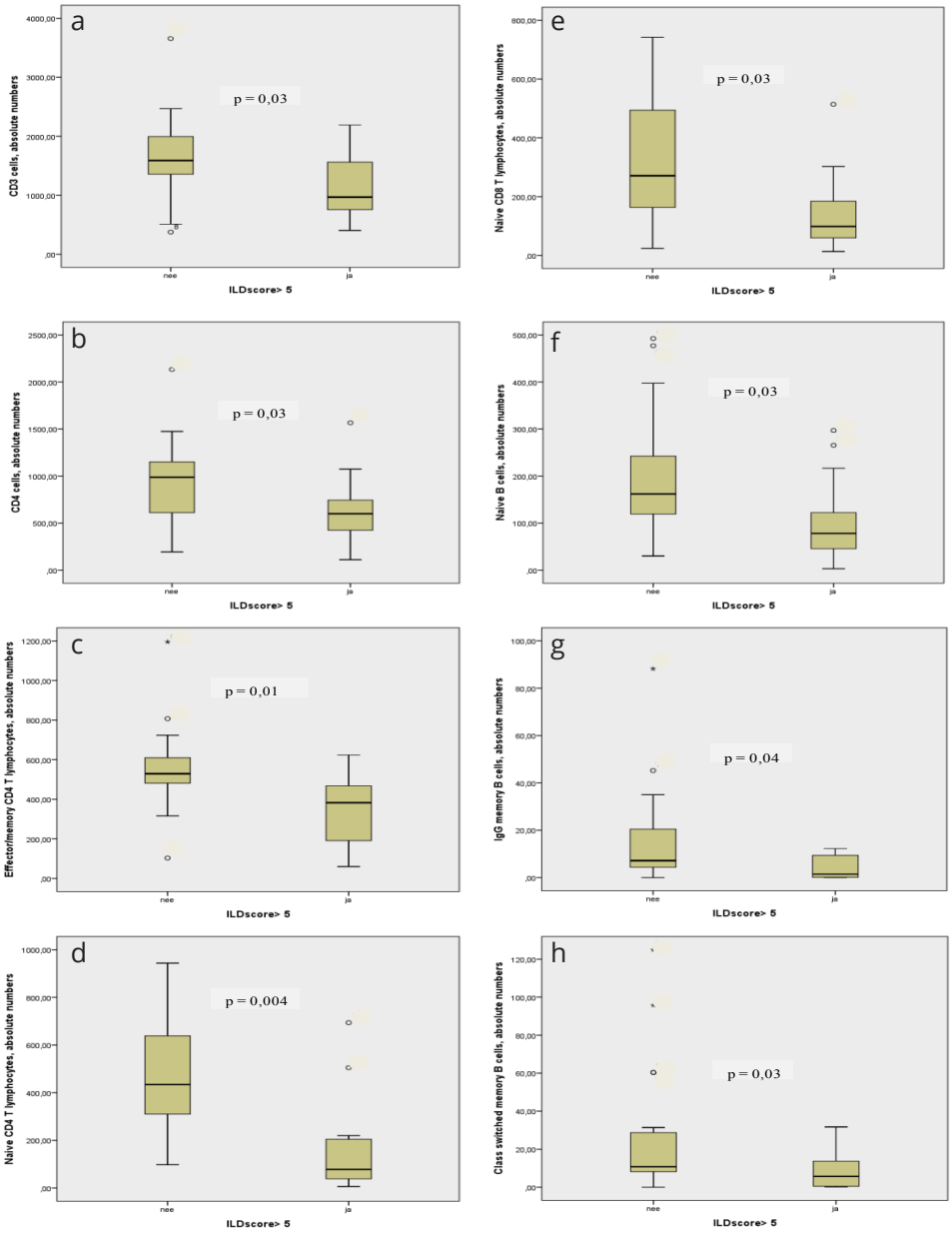

Figure 1 Median count of lymphocytes within T cell and B cell subsut with and without ILD.
CVID: Common variable immunodeficiency disorders; ILD: interstitial lung disease, ILD score >5
*Median absolute counts per mm³
Boxplot: boxes indicates 50th percentile, whispers indicate the 25th and 75th percentile.
Reference values, (all in absolute numbers): from van Gent et al, Clinical Immunology (2009) 133, 95-107. CD3+ T lymphocytes (100–400); CD4 T+ lymphocytes (400–1300); Naive (CD45RA+CD27+) CD4+ T lymphocytes (240-790); Effector/memory CD4+ T lymphocytes (150-500); Naive (CD45RA+CD27+) CD8+T lymphocytes (220-400); Naief (IgM+IgD+CD27-CD10-)B cells (72-257);IgG (IgG+CD27+)memory B cells (2-51); non-Ig class-switched IgM+IgD+CD27+ memory B cells, IgG+CD27+ and IgA+CD27+ memory B cells.

to patients with an ILD score ≤ 5 (601/mm³ vs. 989 mm³; p=0.03). This reduction was mainly related to lower absolute numbers of naïve CD4+ T cells (78 vs. 434 cells/mm³; p= 0.004) and effector/memory CD4+ T cells (383/mm³; vs. 529/mm³; p=0.01). Additionally, the absolute numbers of naïve CD8+ T cells were reduced in the group of patients with significant signs of ILD when compared to the patients without (table 2). Patients with an ILD score >5 also showed lower numbers of naïve B cells and IgG memory B cells when compared to patients with an ILD score ≤ 5 (naïve B cells: 78/mm³ vs. 162/mm³; p=0.03 and IgG memory B cells: 1.5/mm³ vs. 7/mm³; p=0.04). Furthermore, the absolute number of class switched memory B cells were reduced in the patients with an ILD score >5 when compared to the patients with a score ≤ 5 (6/mm³ vs. 11/mm³ respectively; p=0.03). The absolute number of CD4+ T cells and the naïve CD4+ T cells were found to be markers with the highest sensitivity (80% and 82%, respectively) and positive predictive values (60% and 64% respectively) to identify patients at risk for ILD.
No differences were found in the T- and B cell markers between patients with and without significant airway disease scores (table 3) and more specific between patients with and without bronchiectasis.

Gastrointestinal pathology and immunological characteristics

Subtle differences were detected in T- and B cell counts between patients with and without gastrointestinal (GI) pathology (table 4) .Patients with nodular lymphoid hyperplasia (NLH) had lower IgA levels at the diagnosis of CVID than patients without NLH (0.07 g/L vs. 0.5 g/L, p=0.04). Furthermore, median CD3+ T cells were lower in patients with (histological) signs of ileitis or colitis than patients without (697/mm³ vs. 1331/mm³, p=0.03). Although numbers were small, in the eight patients with polyps absolute numbers of naïve B cells and recent bone marrow emigrants (RBE) B cells were lower when compared to the patients without polyps (naïve B cells:47/mm³ vs. 159/mm³, RBE B cells 3/mm³ vs. 22/mm³).

Table 3 Clinical and immunological differences between CVID patients with and without airway disease

n=47	**AD≤7**		**AD>7**		**p**
	n=33	IQR	n=14	IQR	
Age at diagnosis, yrs	29	(16-37)	24	(16-56)	0.9
Time to diagnosis, yrs	10	(5-17)	9	(5-12)	0.5
Age at time of CT scan, yrs	37	(30-52)	37	(25-61)	0.9
IgG trough level during Ig replacement therapy during the last ten years, g/l	8.9	(8.2-9.9)	8.8	(7.4-9.7)	0.4
Respiratory tract Infections, nr of patients (%)					
nr of patients with pneumonia before Ig therapy	8	(24%)	5	(36%)	0.4
nr of patients with pneumonia since Ig therapy	2	(6%)	5	(36%)	**0.009**
CVID related complications, nr of patients (%) ¶ §					
none	16	(49%)	8	(57%)	0.5
Auto-immune disorders[1]	6	(18%)	2	(14%)	0.7
Lymphoproliverative disorders[2]	8	(24%)	5	(36%)	0.8
Enteropathy	6	(18%)	4	(29%)	0.4
Malignancy	3	(9%)	0	0	0.1
NK cells*	210	(124-264)	133	(89-193)	0.1
CD3+ T lymphocytes*	1565	(806-1856)	1381	(952-2037)	0.9
CD4 T+ lymphocytes*	825	(535-1064)	754	(472-1216)	0.8
Activated CD4+ T lymphocytes*	12	(3-20)	10	(8-22)	0.6
Naive CD4+ T lymphocytes*	326	(110-505)	324	(72-695)	0.9
Effector/memory CD4+ T lymphocytes*	512	(391-610)	363	(232-524)	0.1
CD8+ T lymphocytes*	532	(271-772)	530	(339-727)	0.9
Activated CD8+ T lymphocytes*	14	(3-24)	11	(7-24)	0.8
Naive CD8+T lymphocytes*	181	(68-360)	229	(98-442)	0.6
Effector/memory CD8+ T lymphocytes*	236	(143-400)	276	(173-448)	0.9
CD4/8 ratio	1.5	(1.1-2.4)	1.5	(1.1-2.2)	0.8

Table 3 Continued

n=47	AD≤7		AD>7		p
	n=33	IQR	n=14	IQR	
CD19+ B lymphocytes*	204	(84-340)	255	(113-413)	0.5
Naive B lymphocytes*	122	(46-201)	142	(59-289)	0.4
Recent Bone Marrow Emigrants B lymphocytes*	12	(5-35)	19	(6-30)	0.4
IgM memory B lymphocytes*	38	(8-81)	24	(14-58)	0.4
IgG memory B lymphocytes*	5.6	(1.5-14)	5.7	(2-11)	0.3
IgA memory B lymphocytes*	3.9	(2-12)	2.8	(1.5-9)	0.3
Total class switched memory B lymphocytes*	9.8	(3.5-26)	9.6	(4-21)	0.2
Switched memory B lymphocytes ≤ 2% of total B lymphocytes #	4	(17%)	4	(33%)	0,2

CVID: Common variable immunodeficiency disorders; AD: airway disease: AD score >7
NK cells: Natural Killer cells, n= number of patients.
Yrs: years; IQR: interquartile range; g/L: gram/liter
§ Patients could have multiple complications
*Median absolute counts per mm^3 (IQR)
¶ according to Chapel classification (6,7)
# according to EUROclass trial (10).
[1] auto-immune: cytopenia and organ- specific autoimmunity
[2] polyclonal lymphocytic infiltration (defined as unexplained persistent lymphadenopathy. granulomatous disease or hepatosplenomegaly
Reference values, (all in absolute numbers): from van Gent et al, Clinical Immunology (2009) 133, 95-107
CD3+ T lymphocytes (100–400); CD4 T+ lymphocytes (400–1300); CD8+ T lymphocytes(200–700); CD4/CD8 ratio (1.1–3.2);
Activated CD4+ T lymphocytes (2.5-8.5); Naive CD4+ T lymphocytes (240-790); Effector/memory CD4+ T lymphocytes (150-500);
Activated CD8+ T lymphocytes (4-19);Naive CD8+T lymphocytes (220-400); Effector/memory CD8+ T lymphocytes (50-190);
CD19+ B lymphocytes (100-400); Naief B cells (72-257); Recent Bone Marrow Emigrants B cells (6-41);
IgM memory B cells (10-39); IgG memory B cells (2-51); IgA memory Bcells (1-20).

CD19+ B cell compartment: IgD+CD10+CD38++ recent bone-marrow emigrants (RBE), IgM+IgD+CD27-CD10- naive B cells,
non-Ig class-switched IgM+IgD+CD27+ memory B cells, IgG+CD27+ and IgA+CD27+ memory B cells.
CD3+ T cells were divided into CD4+ and CD8+ subsets, and subsequently into CD38+HLA-DR+ activated T cells, CD45RA+CD27+ naïve,
and non-naïve CD45RA+CD27-, CD45RA-CD27+ or CD45RA-CD27- T cells.

Table 4 Clinical and immunological differences between CVID patients with and without gastrointestinal pathology

	NLH no		**NLH yes**		
n=30	n=14	IQR	n=16	IQR	p
Age at diagnosis, yrs	34	(17-59)	23	(12-34)	0.07
Time to diagnosis, yrs	12.5	(4-26)	10.5	(5-15)	0.1
Age at time of endoscopy, yrs	41	(29-62)	31	(23-47)	0.09
IgG trough level during Ig replacement therapy, g/l	9.3	(8.5-10.1)	8.3	(6.9-9.4)	0.2
Serum Ig at diagnosis, g/L					
IgG	4.8	(3.1-5.6)	3.4	(0.9-5.2)	0.1
IgM	0.6	(0.3-0.9)	0.5	(0.2-0.9)	0.5
IgA	0.5	(0.1-1.2)	0.07	(0-0.5)	**0.04**
CVID related complications, nr of patients (%)¶ #					
none	7	(50%)	7	(44%)	0.7
Auto-immune disorders[1]	2	(14%)	5	(31%)	0.2
Lymphoproliverative disorders[2]	2	(14%)	3	(19%)	0.7
Malignancy	2	(14%)	0	0	0.1
NK cells*	173	(127-199)	168	(96-254)	0.6
CD3+ T lymphocytes*	1050	(911-1830)	1267	(888-7812)	0.8
CD4 T+ lymphocytes*	611	(330-1097)	610	(545-866)	0.9
Activated CD4+ T lymphocytes*	6	(2-15)	10	(5-25)	0.2
Naive CD4+ T lymphocytes*	314	(165-638)	189	(88-459)	0.2
Effector/memory CD4+ T lymphocytes*	403	(268-531)	410	(317-497)	0.9
CD8+ T lymphocytes*	557	(349-704)	530	(326-786)	0.7
Activated CD8+ T lymphocytes*	8	(3-13)	9	(4-18)	0.4
Naive CD8+T lymphocytes*	237	(68-514)	132	(100-265)	0.5
Effector/memory CD8+ T lymphocytes*	237	(165-307)	234	(144-390)	0.6
CD4/8 ratio	1.4	(0.8-1.6)	1.3	(0.9-1.7)	0.3
CD19+ B lymphocytes*	204	(56-383)	242	(108-449)	0.5
Naive B lymphocytes*	122	(38-206)	151	(69-342)	0.4
Recent Bone Marrow Emigrants B lymphocytes*	12	(2-34)	18	(4-29)	0.5
IgM memory B lymphocytes*	14	(4-48)	23	(9-49)	0.7
IgG memory B lymphocytes*	5	(1-7)	6	(1.5-15)	0,5
IgA memory B lymphocytes*	3	(1-5)	4	(1-8)	0.5
Total class switched memory B lymphocytes*	9	(6-14)	10	(4-24)	0.3

CVID: Common variable immunodeficiency disorders; NLH: Nodular lymphoid hyperplasia
NK cells: Natural Killer cells
Yrs: years; IQR: interquartile range; g/L: gram/liter
Patients could have multiple complications
*Median absolute counts per mm^3 (IQR)
¶ according to Chapel classification (3,4)
[1] auto-immune: cytopenia and organ- specific autoimmunity
[2] polyclonal lymphocytic infiltration (defined as unexplained persistent lymphadenopathy. granulomatous disease or hepatosplenomegaly

ileitis/colitis no		**ileitis/colitis yes**			**Polyp no**		**Polyp yes**		
n=21	IQR	n=9	IQR	p	n=22	IQR	n=8	IQR	p
29	(14-46)	22	(12-36)	0.5	22	(12-30)	37	(34-56)	**0.02**
11.5	(6.5-19)	8	(3-15)	0.2	10	(5-14)	20	(8-42)	0.1
31	(24-54)	41	(27-58)	0.4	30	(24-40)	53	(46-62)	**0.004**
9.2	(7.7-10.2)	8.3	(6.7-9.2)	0.07	9.2	(7.9-9.8)	8.3	(7-9.9)	0.3
4.7	(2.8-5.4)	2.7	(0.2-5.4)	0.2	4.8	(2.7-5.6)	2.9	(0-3.8)	**0.02**
0.6	(0.3-1.0)	0	(0-0.2)	0.1	0.6	(0.3-0.9)	0.5	(0.1-4.1)	0.06
0.4	(0.03-1.0)	0.2	(0-0.6)	0.2	0.4	(0.02-0.9)	0	(0-0.5)	0.5
12	(57%)	2	(22%)	0.07	10	(46%)	4	(50%)	0.8
4	(19%)	3	(33%)	0.3	5	(23%)	2	(25%)	0.8
4	(19%)	1	(11%)	0.5	3	(14%)	2	(25%)	0.4
2	(9.5%)	0	0	0.3	1	(5%)	1	(12.5%)	0.4
175	(130-228)	125	(46-233)	0.1	159	(112-232)	174	(111-264)	0.5
1334	(975-1940)	967	(594-1267)	0.03	1284	(968-1906)	1108	(808-1638)	0.5
708	(572-1110)	344	(327-601)	0.05	655	(534-1077)	589	(307-834)	0.3
12	(3-21)	3	(3-17)	0.4	9	(2-21)	10	(7-25)	0.3
314	(165-694)	78	(57-227)	0.05	224	(110-638)	165	(76-599)	0.7
412	(391-512)	289	(186-434)	0.6	411	(345-482)	403	(175-541)	0.8
557	(384-846)	380	(266-586)	0.06	531	(329-690)	550	(357-775)	0.8
9	(3-19)	8	(4-14)	0.5	8	(3-16)	9	(6-39)	0.09
237	(130-514)	91	(46-165)	0.06	222	(99-356)	130	(84-408)	0.6
237	(165-307)	185	(82-489)	0.9	234	(151-351)	261	(160-522)	0.3
1.4	(0.8-1.7)	1.4	(0.9-3.5)	0.1	1.5	(1.1-1.7)	1.2	(0.5-1.5)	0.2
219	(132-448)	59	(20-330)	0.3	212	(96-404)	176	(54-1099)	0.4
122	(45-324)	175	(80-247)	0.9	159	(122-299)	47	(31-151)	**0.02**
14	(6-34)	25	(9-37)	0.6	22	(12-35)	3	(2-21)	**0.03**
15	(7-43)	35	(6-100)	0.8	15	(9-42)	40	(2-138)	0.1
5	(2-9)	11	(1-19)	0.2	5	(3-12)	5	(0-9)	0.3
3	(1-5)	5	(0.7-8)	0.8	3	(1-4)	4	(0-12)	0.4
9	(5-14)	16	(2-26)	0.3	8	(6-14)	12	(0.7-16)	0.8

Reference values, (all in absolute numbers): from van Gent et al, Clinical Immunology (2009) 133, 95-107 CD3+ T lymphocytes (100–400); CD4 T+ lymphocytes (400–1300); CD8+ T lymphocytes(200–700); CD4/CD8 ratio (1.1–3.2); Activated CD4+ T lymphocytes (2.5-8.5); Naive CD4+ T lymphocytes (240-790); Effector/memory CD4+ T lymphocytes (150-500); Activated CD8+ T lymphocytes (4-19);Naive CD8+T lymphocytes (220-400); Effector/memory CD8+ T lymphocytes (50-190); CD19+ B lymphocytes (100-400); Naief B cells (72-257); Recent Bone Marrow Emigrants B cells (6-41); IgM memory B cells (10-39); IgG memory B cells (2-51); IgA memory Bcells (1-20).

CD19+ B cell compartment: IgD+CD10+CD38++ recent bone-marrow emigrants (RBE), IgM+IgD+CD27-CD10- naive B cells, non-Ig class-switched IgM+IgD+CD27+ memory B cells, IgG+CD27+ and IgA+CD27+ memory B cells.
CD3+ T cells were divided into CD4+ and CD8+ subsets, and subsequently into CD38+HLA-DR+ activated T cells, CD45RA+CD27+ naïve, and non-naïve CD45RA+CD27-, CD45RA-CD27+ or CD45RA-CD27- T cells.

Discussion

To identify patients at risk for pulmonary and gastrointestinal complications, we compared T- and B cell subpopulations in a group of CVID patients with well-defined gastrointestinal- and pulmonary pathology. The key finding of this study is that CVID patients with significant signs of ILD have a distinct cellular distribution of T- and B cell subpopulations when compared to patients without ILD. In ILD patients the absolute numbers of CD4+ T cells, naive CD4+ T cells, and naïve and switched memory B cells were markedly lower. Furthermore, ILD was associated with the presence of autoimmunity. We also identified less pronounced abnormalities in patients with gastrointestinal pathology, such as lower IgA levels in patients with nodular hyperplasia and lower CD3 counts in patients with inflammatory colitis. These results are of value for the development of future clinical screening protocols, as early and accurate detection of pulmonary and gastrointestinal complications is important to prevent mortality associated with end organ disease (8;23).

As of yet, only a few studies have explored T cell phenotype in adult CVID patients with pulmonary disease. An earlier study in children showed different T cell abnormalities in children with ILD when compared to our study. In that particular study, the differences between children with and without ILD were found in the CD8+ T cell compartment only. (41). Evidently, differences in the T cell compartment exist between age-groups, as the memory subset will gradually increase during aging. Furthermore, in general children experience more viral infections, and therefore an increased activation pattern can be expected in the CD8+ T cell compartment. A decreased thymic output on the one hand and a decreased number of regulatory T cells (not investigated in this study) on the other hand have been established in CVID patients (1;45) and could be an explanatory mechanism for the auto inflammatory conditions. Earlier (clinical) studies have shown that ILD is associated with the presence of splenomegaly (44;46), lymphadenopathy (47) and autoimmune manifestations (44) which is in line with our findings. Furthermore, ILD has been associated with lower number of memory B

cells, previously established in children (41). Lower number of memory B cells has also been described as a risk factor for CVID related non-infectious complications (10-12).

It is known, that recurrent infections – which are clearly related to a failure in B-cell differentiation leading to reduced serum immunoglobulin (Ig) levels and an abnormal antibody response - can evoke exacerbations of inflammatory diseases (48).

In patients with signs of airway disease (AD) we could not detect differences in the B- and T cell compartment when compared to patients without AD. It therefore seems that AD develops mainly due to the cumulative effect of recurrent infections and subsequent deterioration of lung tissue. However, in earlier studies bronchiectasis was shown to be related to a reduced number of B memory cells (17;49;50).

The gastrointestinal tract is the largest immune organ in the body and serves as an important barrier to ingested foreign antigens; IgA being the major immunoglobulin in the gut. It can therefore be expected that defects in the immune system in CVID patients may result in considerable gastrointestinal pathology. Different explanations may justify the occurrence of these conditions in patients with antibody deficiencies despite appropriate Ig replacement therapy. First, IVIg therapy only substitutes IgG, whilst IgA (the major secretory antibody at mucosal surfaces) is not replaced. Other possible explanations could be on-going inflammation after treated infections (51) and concurrent T-cell defects (3;51).

In our study however, only subtle immunological differences were detected which may be due to the limited number of patients in the current study. Patients with signs of ileitis or colitis had lower CD3+ T cells which is in line with a previous study (52). An earlier study failed to show this correlation (52). In another study with a more clinical basis, changes in the number of switched memory B cells was indeed associated with intestinal disease such as malabsorption syndrome and chronic noninfectious diarrhea (49).

This study has several limitations. First, the number of patients was relatively small, and therefore our findings require confirmation in larger studies. Second, a clear threshold (cutoff) for the immune-

phenotypic markers could not be identified. Third, to develop a prediction model to define patients are at risk for pulmonary or gastrointestinal complications we need prospective studies.

In conclusion, we demonstrated that CVID patients with (preclinical) signs of ILD on thin slice CT have a distinct cellular distribution of T and B cell subpopulations when compared to patients without. The absolute number of the total CD4+ T cells count and the naïve CD4+ T cells and switched memory B cells might be candidate immunological markers to identify patients at risk for ILD and contribute to tailored monitoring of these patients. However, larger and prospective studies are required to determine cut off values for T- and B cell markers to discern patients at risk for ILD and to ascertain the optimal follow up frequency aimed at preventing morbidity and mortality in CVID patients.

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7

Summary, general discussion and future perspectives

Common variable immunodeficiency (CVID) is the most common and most diverse primary antibody deficiency. The heterogeneous group consists of patients with variable (recurrent) infections and non-infectious complications such as autoimmunity, lymphoproliferative (granulomatous) disease, chronic lung disease, enteropathy, lymphoma and other malignancies. The largest problem for CVID patients is the development of the non-infectious complications that are associated with excess morbidity and mortality due to organ dysfunction and failure (1-3). Post-diagnosis survival is estimated 65% for the first 6.5 years and 45% after 14 years which is mainly caused by progressive end-organ disease, mainly in lungs and gut (1;4). Although CVID patients share clinical features (e.g. recurrent infections) they differ greatly in their clinical course and prognosis (5), probably due to the different underlying immune pathogenic mechanisms which are largely unknown. Therefore, recognition of the specific patients at risk for CVID related complications by establishing risk factors may accelerate diagnosis and improve follow-up and treatment of patients. The CVID related complications have been used to classify patients into clinical phenotypes with varying prognoses (2;5). Furthermore, classifications of patients have been made by the analysis of B- (6-8) and T cell compartments by flowcytometry (9-12) in combination with complications. Worldwide research is currently focusing on differentiating between the various disorders and genetic causes in order to dissect the syndrome of common variable immunodeficiency and to improve the outcome of these patients.

In the current thesis we set out to identify clinical and therapeutic aspects and B- and T cell parameters that might predict which patients are prone to complications in order to improve clinical follow up and treatment of CVID patients.

The studies in this thesis describe the treatment options in CVID and its complications (review), the prevalence of the complications in a cohort of adult patients, and the prevalence of pulmonary and gastro intestinal pathology and possible related risk-factors, such as clinical parameters, treatment and immunological parameters.

I. Summary of the main findings

In ***chapter 2*** we addressed all aspects of immunoglobulin (Ig) therapy. The current standard of care in CVID is immunoglobulin (Ig) replacement therapy given at frequent intervals for life. Both intravenous immunoglobulin (IVIg) and subcutaneous immunoglobulin (SCIg) treatment appear to be safe, with comparable efficacy. Infusion-related adverse reactions have been reduced considerably in recent years owing to improved manufacturing processes. The advantages of SCIg are more stable IgG levels, the absence of serious systemic adverse events and more flexibility in the patient's social life. The beneficial effect of Ig replacement therapy on frequency and severity of infections and survival rates of CVID patients is undisputable. However, Ig replacement therapy does not appear to protect against or treat the non-infectious inflammatory complications. In general, Ig replacement therapy is indicated in (i) patients with IgG levels <2 g/L, (ii) patients with documented frequent infections and a specific antibody deficiency with IgG levels between 2 g/L and 5 g/L or (iii) patients with IgG levels >5 g/L but severe and recurrent infections and a specific antibody deficiency (13). Based on various studies, a 2006 review by members of the Primary Immunodeficiency Committee of the American Academy of Allergy, Asthma and Immunology (14) recommends that IgG trough levels should be >5 g/L for patients with agammaglobulinaemia and 3 g/L greater than the initial IgG level for patients with CVID. Based on the fact that an IgG trough level of >5 g/L clearly reduces the infection rate, and IgG trough levels >8 g/L might improve chronic pulmonary outcome, we aim for an IgG trough level >8 g/L in our practise and for patients with bronchiectasis even higher (> 9 g/L).

In ***chapter 3*** the spectrum of illness is described of patients with antibody deficiency syndromes in a retrospective study. Recurrent infections (mainly respiratory) were the key presentation of CVID patients (90%) and the number of patients with repetitive infections diminished (70-100% reduction) after the start of immunoglobulin (Ig) therapy, although this effect was considerably less for acute and chronic sinusitis, which

was still present in 60% of patients. The development of chronic pulmonary disease and non-infection related complications were not halted by adequate Ig therapy. The prevalence of bronchiectasis increased despite Ig therapy (10% of patients prior to diagnosis of CVID to 29% during follow up) and the non-infectious disease complications, mainly autoimmune disease, lymphoproliverative conditions and gastrointestinal disease were present in 30% of CVID patients at the time of diagnosis which increased to 51% of patients during follow up. We detected associations between immunological parameters within the B and T cell compartment and the specific complications related to CVID. A low percentage (≤2%) of class switched memory B cells was associated with autoimmune disease as well as with splenomegaly and granulomatous disease. Furthermore, the patients with any complication and >2% of class switched memory B cells had lower median numbers of class switched memory B cells when compared to patients without complications. In patients with one or more complications the absolute numbers of CD3, CD4 + T cells, naive CD4+ T cells and naive CD8+ T cells were significantly lower. Patients with autoimmune disease had significant lower absolute numbers of CD3, CD4 and CD8 + T cells when compared to patients without autoimmune disease. We found a considerable delay in the diagnosis of CVID (median 10 years, IQR 5-16 yrs.). Moreover, the median time to diagnosis in the group of patients with one complication or more prior to diagnosis was seven years longer ($p<0.05$) in comparison to the group of patients without any complications. In comparison, IgG subclass deficiency and SADNI patients did not develop any complications during follow-up. The effect of therapy (IgG therapy or antibiotic prophylaxis) in these patients clearly diminished the occurrences of pneumonia.

In ***chapter 4*** we describe the prevalence and spectrum of gastrointestinal pathology by endoscopic screening in a cross sectional study in 30 CVID patients. We found that gastrointestinal pathology is frequent in CVID patients regardless of (the presence or absence of) symptoms and despite Ig therapy. However, in this small cohort we did not detect any malignancies. We did find Helicobacter pylori-

associated gastritis as well as adenoma, which are established risk factors for malignancies. In 25 (83%) of the 30 CVID patients new endoscopic and/or histological abnormalities were detected of which 30% had therapeutic consequences, including esophagitis, *Helicobacter pylori*-associated gastritis and adenoma. In only 44% of CVID patients symptoms could be correlated with endoscopic findings. Moreover, 40% of the asymptomatic patients were found to have gastrointestinal abnormalities, such as *Helicobacter pylori*-associated gastritis which is important in the light of the increased incidence of gastric carcinoma in CVID.

In ***chapter 5*** we describe the prevalence and spectrum of (pre-clinical) chronic pulmonary disease in 47 CVID patients in a cross sectional study using thin slice Computed Tomography (CT) scans and pulmonary function testing. Subtle pre-clinical pulmonary abnormalities were detected by CT in virtually all CVID patients and significant airway disease (AD score >7) or interstitial lung disease (ILD score >5) was present in 30% and 34% of patients, respectively. Bronchiectasis was present in as much as 64% of patients and 75% of the patients were found to have airway wall thickening, which is known to precede the development of bronchiectasis. Airway disease was correlated to the occurrence of pulmonary infections. The correlation between the CT scores and the results of pulmonary function testing in our study was poor, indicating that pulmonary function testing is a poor predictor of early pulmonary abnormalities.

Both chronic pulmonary and gastrointestinal disease have been associated with excess morbidity and early mortality in CVID patients. Therefore, early detection and monitoring of progression of such conditions is essential. In ***chapter 6*** we set out to find immunological parameters to identify patients at risk for gastrointestinal- and pulmonary complications by analysing peripheral blood B and T lymphocyte subsets in CVID patients with well-defined gastrointestinal- and pulmonary pathology and compared the results to CVID patients without these complications. The key finding was that CVID patients

with significant signs of ILD had a distinct cellular distribution of T and B cell subpopulations when compared to patients without ILD. In ILD patients the absolute numbers of CD4+ T cells, naïve CD4+ T cells, the naïve CD8+ T cells, and naïve and memory B cells were markedly reduced. However, clear cut-off values could not yet be identified. Furthermore, ILD was associated with the presence of autoimmune disease. Airway disease (AD) could not be correlated to specific T- and B cell phenotypes. We identified less pronounced abnormalities in lymphocyte distribution in patients with signs of gastrointestinal disease. Most importantly patients with nodular hyperplasia had lower IgA levels and patients with inflammatory colitis had lower numbers of total T lymphocytes.

II. Discussion

1. The prevalence of complications in antibody deficiencies

Our research has shown that the clinical spectrum in CVID patients is divers and that the development of infectious and non-infectious complications continues despite Ig therapy (*chapter 3, 4* and *5*). The beneficial effect of Ig replacement therapy on short-term complications (acute respiratory infection rate and antibiotic usage) is undisputable (*chapter 2*). However, conflicting evidence exists on the beneficial effect on long term complications and therefore its effect on the occurrence of non-infectious complications and end organ damage remains still questionable.

We have showed that the prevalence of structural airway disease (bronchiectasis) increased during follow-up (*chapter 3* and *5*) despite adequate IgG trough levels and despite the reduction of respiratory infections. Few studies have evaluated the effect of Ig treatment on the evolution of pulmonary damage such as bronchiectasis and the data is conflicting. One prospective study showed that some pulmonary improvement was demonstrated in a group of patients with chronic pulmonary disease (CPD), as the forced expiratory volume in 1 s (FEV_1) (as a percentage of the predicted value) increased from 54% to 61%. The median IgG trough level in this CPD group was lower compared to

the group without CPD (7.2 ± 1.4 g/L (range 5.7–9.8 g/L) vs. 8.5 ± 1.6 g/L (range 6.0–11.6 g/L)). Also to achieve the desired trough levels, patients with chronic pulmonary disease (CPD) needed higher doses of IVIg than those without CPD (15). A recent study (16) confirmed that CVID patients with bronchiectasis need higher IgG replacement doses to achieve similar IgG trough levels (0.70 ± 0.29 g/kg/month vs. 0.53 ± 0.20 g/kg/month). In contrast, two prospective studies showed that despite IVIg treatment and the significant reduction of acute respiratory infections, progression of CPD and chronic sinusitis continued (17;18).

In line with other cohort studies (2;17;19) the prevalence of non-infectious complications increased despite Ig therapy (*chapter 3,4* and *5)*. It is known that the therapeutic benefit of Ig therapy is not limited to antibody replacement but has also shown to be beneficial in autoimmune disease (20). However, the doses used in those treatments are usually much higher than those used for Ig replacement therapy and the mechanism of its immunomodulatory and anti-inflammatory effects remains unclear (20). It is known however that recurrent infections - which are clearly related to insufficient IgG trough levels in CVID - can evoke exacerbations of inflammatory diseases (21).

Common complications are autoimmune, gastrointestinal disease and lymphoproliferative disease (2;17;19). This is probably due to the combined immune dysregulation in the B and T cell compartment in CVID patients. Therefore the contribution of immune dysregulation in patients with CVID cannot be underestimated (9). In our studies, patients with autoimmunity or inflammatory organ disease were found to have a distinct cellular distribution of T and B cell subpopulations. This finding was comparable to other reports (6-9;22). The absolute numbers of CD4+ T cells, naive CD4+ T cells, and naïve and switched memory B cells were significantly lower in patients with autoimmunity or inflammatory organ disease. The absolute number of the total CD4+ T cells count and the naïve CD4+ T cells and switched memory B cells might be candidate immunological markers to identify patients at risk for complications and could contribute to tailored monitoring of these patients. However, larger and prospective studies are required to determine cut off values for T and B cell markers to discern patients at risk for these complications.

Until genetic pathways are identified, the CVID syndrome remains a clinical description of a heterogeneous population of patients that share clinical features but differ in clinical course and prognosis. This is probably due to the different underlying immune pathogenic mechanisms. We conclude that the development of non-infectious complications and end organ damage continues and IgG trough level is not a reliable surrogate marker for the success of therapy concerning the long term complications. The assessment of risk factors may help to identify patients at risk for the development of the specific CVID related complications and the development of screening programs.

2. Screening for CVID related complications

Both chronic pulmonary and gastrointestinal disease have been associated with excess morbidity and early mortality in CVID patients (1;2;17). Therefore, early detection and monitoring of progression of such conditions is essential.

Screening for gastrointestinal complications

Gastrointestinal disease develops in CVID patients despite Ig therapy. Progression of chronic gastrointestinal diseases may still occur in patients with CVID since IgG substitution will only substitute IgG, while IgA and IgM, the major secretory antibodies at mucosal surfaces are not replaced, and secondly, immune dysregulation and T cell abnormalities may contribute to the development of gastrointestinal disease. In our study patients with nodular lymphoid hyperplasia (NLH) had indeed lower IgA levels compared to patients without NLH.

We showed that in our cohort of CVID (*chapter 4)* the prevalence of NLH patients was substantially higher (53%) than that reported in the literature (8%–18% in CVID (19;23-26) and 0.1%–1.6% in adults without primary antibody deficiency (27-29)). It has been suggested that NLH is a risk factor for both intestinal and extra intestinal lymphoma (30), but it seems rare (31;32). Therefore, screening or follow up for NLH is not warranted.

The risk of cancer, such as gastric cancer, in patients with CVID is increased; therefore, endoscopic screening should mainly be focussed on the detection pre-maligne gastric conditions and the detection

of colorectal adenoma. *Chapter 4* describes that the presence or absence of symptoms cannot be used to select patients for endoscopic screening.

Helicobacter pylori infection is a risk factor for gastric cancer in the general population and probably in patients with CVID (33;34). *Helicobacter pylori*-associated gastritis was found in three of 30 CVID patients (10%), who were all asymptomatic. Previous studies have reported a prevalence of HP gastritis of 8-41% in CVID patients (23;35). Thus, screening for *Helicobacter pylori*-associated gastritis, either by gastroscopy or urea breath testing in patients with CVID seems appropriate given the frequency of *Helicobacter pylori*-associated gastritis.

Colorectal polyps are generally asymptomatic however, adenomas should be eradicated because of the risk of malignant progression and polyp surveillance has shown to prevent death from colorectal cancer. In *chapter 4* we showed that higher age was found to be a risk factor for adenomatous polyps (53 years (IQR 46-61 yrs.) vs. 26 years (IQR 23-32 yrs.) which is in line with data from the general population (18% in patients aged 50 years, increasing thereafter) (36). Therefore, we feel that screening for adenoma in patients with CVID can be performed following the same screening programs offered to the general population, although numbers in this study may be too small to draw firm conclusions. Larger and prospective studies with repeated endoscopies are needed to determine the frequency of endoscopic screening. Until then we propose for adult patients to initially perform gastro- and colonoscopy at the diagnosis of CVID. Subsequent gastroenterological follow-up will depend upon the type and severity of the findings similar to the general population. In case of normal findings with gastroscopy and colonoscopy, we propose to repeat the gastroscopy every 3-5 years and colonoscopy every 5 years. The follow-up time intervals are only indicative, so symptomatology and risk factors should be taken into account when determining follow-up intervals for individual patients. Prospective and larger studies are needed to establish if and how often repetitive screening is warranted.

Screening for pulmonary complications

We showed that airway disease is correlated to pulmonary infections (*chapter 5*); however, in our analysis 17 of the 30 patients with bronchiectasis had never been diagnosed with pneumonia, therefore infections might have occurred subclinical. Factors such as age, duration of disease or time to the diagnosis of CVID did not differ between the groups of patients with and without airway disease. In combination with the fact that our patients with and without airway disease did not differ in IgG trough level and also the fact that the median IgG trough in the last ten years was adequate (8.9 g/L, range 7.7- 9.9 g/L), we may conclude that pulmonary damage continues and IgG trough level is not a reliable surrogate marker for the success of therapy concerning the long term pulmonary complications. In the continuing process of pulmonary damage, a mucociliary clearance defect could play a role as mucociliary clearance is the first-line of defence against pathogenic microorganisms and disturbance of this process due to airway damage may result in bronchiectasis (37;38). Therefore, infections may occur subclinical and prescription of prophylactic antibiotics might contribute to prevent progression of bronchiectasis. Furthermore, chronic inflammatory dysregulation has been suggested to be associated with bronchiectasis (39;40). However, in our and other studies patients with and without airway disease did not differ in their B- and T cell compartments.

In our study, interstitial lung disease was associated with the presence of autoimmunity and distinct cellular distribution of T and B cell subpopulations. The absolute number of the total CD4+ T cells count and the naïve CD4+ T cells and switched memory B cells might be candidate immunological markers to identify patients at risk for ILD and contribute to tailored monitoring of these patients. However, no clear cut off values could be determined based on our results probably due to under powerment of the study.

Repetitive CT scanning with standardized scoring systems are reliable and reproducible tools to monitor progression of pulmonary abnormalities and to identify patients at risk, but no consensus has yet been reached about the frequency of CT scanning. In the current

practice the time interval for CT evaluation ranges from 1 to 5 years. Alternatively, annual pulmonary function testing has been suggested (17;41;42), however, in our (and other) studies abnormalities on the CT scan were not clearly related to abnormal pulmonary function testing, indicating that pulmonary function testing is a poor predictor of early pulmonary abnormalities and that thin slice CT is a more sensitive way to detect early pulmonary disease. We would suggest for adult patients to perform an initial HRCT scan at the diagnosis of CVID. In the follow up -based on our studies- we would suggest bi- or tri-annual CT screening of CVID patients with:
(I) active autoimmune disease or other non-infectious inflammatory disease;
(II) patients with on-going frequent lower respiratory tract infections;
(III) patients with a significant AD score (>7) or ILD score (>5) on initial thin slice CT scanning; and
(IV) for other CVID patients CT screening once per five years might be sufficient.
Identifying patients at risk for ILD by using B- and T cell phenotyping might be promising, however, larger and prospective studies are required to determine cut off values for T and B cell markers to discern patients at risk for these complications.

Summary of the proposed screenings protocol for lung- and gastrointestinal complications in adult CVID patients

(I) Initial HRCT of the lungs and gastro- and colonoscopy at the diagnosis of CVID
(II) Subsequent pulmonary follow-up:
bi- or tri-annual HRCT screening of CVID patients with:

1. active autoimmune disease or other non-infectious inflammatory disease;
2. patients with on-going frequent lower respiratory tract infections;
3. patients with a significant AD score (>7) or ILD score (>5) on initial thin slice CT scanning; and
4. for other CVID patients CT screening once per five years might be sufficient.

(III) Subsequent gastrointestinal follow-up:
Gastroscopy: every 3-5 years, unless there are signals which require immediate follow up;
Colonoscopy: every 5 years, unless there are signals which require immediate follow up;
Subsequent gastroenterological follow-up will depend upon the type and severity of the findings similar to the general population and symptomatology and risk factors should be taken into account when determining follow-up intervals for individual patients.

Awareness
Finally, in *chapter 3* we found a considerable diagnostic delay for CVID patients, especially in those patients who were dominated by non-infectious complications. Failure to diagnose CVID and therefore delaying the start of adequate therapy for specific conditions can cause considerable morbidity. The median time to diagnosis for the CVID patients in our cohort (10 years) was comparable to previously reported (3-15 years) (2;17;19;43-45). Therefore, reducing the diagnostic delay will contribute to early detection of the (non)-infectious complications and to ascertain the start of the optimal therapy. And although Ig therapy seemed to have little effect on the development of new complications, specific disease related therapy would be started in an earlier stage of the disease which could affect morbidity and mortality. It remains important to increase awareness among doctors for the variable clinical presentations and manifestations of CVID. The European Society for immunodeficiencies (ESID) (46) has issued warning signs when to suspect a primary immunodeficiency which have shown to be effective tools to recognize primary immunodeficiency provided that they are applied broadly by general practitioners, pulmonary physicians, internal medicine doctors and ear, nose and throat physicians. Therefore education on this subject is necessary.

III. Future perspectives

Larger and prospective studies are needed to confirm the immunological and clinical risk factors that identify CVID patients at risk for complications. Furthermore, unravelling the cellular mechanism and causes of complications might lead us to better treatment interventions. The setup of national and international CVID registries in order to expand CVID cohorts will improve research in CVID to identify subgroups with the possibility to dissect the different underlying immune pathogenic mechanisms. Furthermore, prospective IgG dosing studies are required to recognize any benefit for non-infectious complications.

Continued longitudinal observational cohort studies with repeated gastrointestinal endoscopies are necessary to determine the optimal frequency of endoscopic screening. Repetitive screening with HrCT and pulmonary function in longitudinal observational cohort studies will be necessary to ascertain the optimal follow up frequency of the pulmonary abnormalities, and for evaluation of therapeutic actions aimed at preventing the progression of pulmonary morbidity in CVID patients.

In respect to awareness, we aim to investigate the prevalence of primary antibody deficiencies in selected severe COPD and asthma cases and also evaluate if the ESID PID warnings signs would have retrospectively predicted their presence.

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8

Nederlandse samenvatting
Dankwoord
Curriculum Vitae

Introductie

Het immuunsysteem is opgebouwd uit een groot aantal cellen en moleculen, die door onderlinge interactie zorgdragen voor een adequate respons bij o.a. infecties. Lymfocyten maken deel uit van het immuunsysteem en ontstaan uit stamcellen in het beenmerg om uiteindelijk uit te rijpen tot effector cellen. Een deel ontwikkelt zich in het beenmerg via een aantal stappen tot B-lymfocyten, terwijl een ander deel zich in de thymus verder ontwikkelt tot T-lymfocyten. De cellulaire afweer (T- lymfocyten) is o.a. gericht tegen intracellulaire micro-organismen (virussen, bacteriën). Onder de humorale afweer verstaan we B-lymfocyten en de immunoglobulinen (Ig, antistoffen). De immunoglobulinen (IgA, IgM, IgG, IgD en IgE) worden geproduceerd door de B- lymfocyt (plasmacellen) als reactie op lichaamsvreemde stoffen (antigenen) zoals bacteriën. Door de interactie met immunoglobulinen worden de antigenen onschadelijk gemaakt. Naast de antilichaam-producerende plasmacellen ontstaan ook B-geheugencellen.
Patiënten met een primaire immuundeficiëntie hebben ten gevolge van een defect in één of meerdere genen een stoornis in het afweersysteem. Hierdoor zijn zij extra vatbaar voor infecties.
Primaire antistofdeficiënties, ziektebeelden waarin verminderde of zelfs afwezige productie van immunoglobulinen (ten gevolge van B- lymfocyt dysfunctie) op de voorgrondtreden, vertegenwoordigen hierin de grootste groep (50-70%). Het spectrum van deze primaire antistofdeficiëntie omvat milde en de meest voorkomende fenotypes zoals IgA deficiëntie, IgG-subklasse deficiëntie en “selective antibody deficiency with normal immunoglobulins” (SADNI). Maar ook meer ernstig verlopende ziektebeelden als common variable immunodeficiency (CVID) en X-gebonden agammaglobulinaemia (XLA).

Common variable immunodeficiency (CVID)

CVID is de meest heterogene aandoening in de groep van primaire antistofdeficiënties. En hoewel CVID patiënten overeen komen in sommige klinische kenmerken (recidiverende infecties), verschillen ze onderling sterk in de leeftijd van presentatie van de eerste

ziekteverschijnselen, het klinische beloop en de prognose. Dit wordt waarschijnlijk veroorzaakt door de verschillende onderliggende immunologische defecten. Bij CVID leidt een gestoorde B-lymfocyt ontwikkeling tot een verminderde immunoglobuline (IgG, IgA e/o IgM) aanmaak. Daarnaast kan er sprake zijn van verschillende T-lymfocyt defecten. Er is een aantal genetisch of immunologische defecten beschreven, echter bij de meeste CVID patiënten is er (nog) geen genetisch defect vastgesteld. CVID patiënten presenteren zich doorgaans met recidiverende luchtweg infecties, maar ook andere infecties komen, zoals maagdarm infecties voor. De behandeling van CVID patiënten bestaat uit het intraveneus of subcutaan toedienen van beschermende antistoffen (immunoglobulinen (Ig) therapie) en eventueel antibiotica ter profylaxe. Dit leidt tot een verminderd optreden van infecties en een goede kwaliteit van leven. Echter, een subgroep van CVID patiënten ontwikkelt ook niet-infectieuze aandoeningen. Voorbeelden van deze CVID gerelateerde complicaties zijn auto-immuunziekten en lymfoproliferatieve afwijkingen (bijv. granulomen in de lever, longen, lymfeklieren, of de huid), vaak in combinatie met een vergrote milt en vergrote lymfklieren. Verder hebben de CVID patiënten frequent maagdarm aandoeningen en een verhoogd risico op kanker (lymfklierkanker en maagdarm kanker). De longen en het maagdarmkanaal zijn het meest frequent aangedaan bij CVID patiënten. Chronische longziekten kunnen bij CVID worden opgedeeld in structurele luchtweg afwijkingen en interstitiële longziekte (ILD). Een voorbeeld van de structurele luchtweg afwijkingen zijn bronchiëctasieën, meestal het gevolg van recidiverende luchtweginfecties. Bronchiëctasieën zijn chronische, abnormale verwijdingen van de bronchiën door beschadiging van de bronchuswand waardoor slijm niet goed wordt afgevoerd en bacteriën de kans zich er gemakkelijk te nestelen en een chronische ontsteking te veroorzaken. Immunoglobuline therapie en profylactische antibiotica zijn effectief gebleken in het voorkomen van acute infecties en zouden dus indirect deze structurele luchtweg afwijkingen (of de verergering ervan) kunnen voorkomen. Interstitiële longziekte (ILD) omvat onder andere granulomateuze longziekte, lymphoide

interstitiele pneumonie, en lymphoproliverative afwijkingen. De behandeling bestaat uit steroïden of in zeldzame gevallen met andere immuunmodulerende therapie. Darmaandoeningen (bijv. chronische diarree en malabsorptie) worden ook regelmatig gezien zonder dat er sprake is van een darminfectie. Oorzaken hiervan zijn onder andere auto-immuun ziekten (pernicieuze anemie), chronische inflammatoire aandoeningen (atrofische gastritis, vlokatrofie, colitis) en nodulaire lymfoïde hyperplasie. Verder bestaat er een relatief hoog risico op maagdarm kanker bij CVID patiënten ten opzichte van de algemene populatie. Alle bovengenoemde aandoeningen zijn te wijten aan de onderliggende ontregeling van het immuun systeem zoals is beschreven bij CVID patiënten en zijn geassocieerd met verhoogde morbiditeit en mortaliteit.
Wereldwijd onderzoek is momenteel gericht op de pathogenese en de genetische analyse om het syndroom CVID te kunnen ontleden. De ontwikkeling van CVID classificatie systemen op basis van klinische- en immunologische kenmerken heeft tot meer inzicht geleid in de heterogene groep van CVID patiënten. De classificaties kunnen ook waardevol zijn om risico patiënten voor CVID gerelateerde complicaties te herkennen of variabelen te definiëren die voorspellend zouden kunnen zijn voor de ontwikkeling van complicaties, met als doelstelling een verbetering van de follow-up en behandeling van deze patiënten.

IgG subklasse deficiëntie en Selectieve antistof deficiëntie met normale immunoglobulinen (SADNI)

Een klinisch significante IgG subklasse deficiëntie wordt gedefinieerd als recidiverend luchtweginfecties met een verlaging van een of meer van de IgG subklassen maar een normaal totaal IgG gehalte. SADNI is gedefinieerd als het optreden van recidiverende luchtweginfecties bij een abnormale reactie op polysacharidevaccinatie, in aanwezigheid van een normaal serum-IgG (en subklasse)gehalte. Levensbedreigende bacteriële infecties met ernstige complicaties en restverschijnselen zoals bronchiëctasieën zijn bij IgG-subklasse deficiëntie en SADNI een zeldzaam verschijnsel. Niet-infectieuze complicaties zijn niet eerder vastgesteld. Patiënten met een IgG-subklasse deficiëntie en SADNI worden vaak

profylactisch behandeld met antibiotica. Als dit onvoldoende resultaat heeft, kan toediening van immunoglobulinen worden overwogen.

Agammaglobulinemie

X- linked agammaglobulinemie (XLA) is de langst bekende, overigens wel zeldzame immuundeficiëntie. XLA wordt veroorzaakt door een mutatie in een gen (Bruton tyrosine kinase) wat essentieel is voor de B- cel ontwikkeling en wat leidt tot een blokkade van de ontwikkeling van B-lymfocyten in het beenmerg en de daaropvolgende deficiëntie van alle immunoglobuline typen. De T-lymfocyt-gemedieerde immuniteit is intact. Vóór het tijdperk van immunoglobuline therapie overleefden slechts weinig of geen XLA patiënten de (vroege) kindertijd. Levenslange behandeling met immunoglobulinen therapie kan de verhoogde gevoeligheid voor infecties grotendeels ondervangen en heeft de prognose voor XLA patiënten aanzienlijk verbeterd. XLA wordt gekenmerkt door ernstige en recidiverende, voornamelijk bacteriële, infecties, die optreden vanaf de tweede helft van het eerste levensjaar na het verdwijnen van maternale IgG-antistoffen. Infecties van de luchtwegen worden vaak gecompliceerd door de ontwikkeling van structurele luchtweg- en longaandoeningen (bronchiëctasieën, fibrose). Tevens is er het voorkomen van kanker gerapporteerd, waaronder darmkanker. Aandoeningen zoals auto-immuun- of lymfoproliveratieve ziekten zijn zeldzaam.

Dit proefschrift

Dit proefschrift beschrijft studies die we hebben uitgevoerd naar de verschillende klinische aspecten van voornamelijk CVID patiënten. We hebben naast het bepalen van de prevalentie van de verschillende CVID gerelateerde complicaties, ook onderzocht wat de relatie is tussen diverse klinische parameters, therapeutische maatregelen en B- en T-lymfocyt karakteristieken en het optreden van de CVID gerelateerde complicaties. De longen en het maagdarmkanaal zijn de belangrijkste focus geweest in dit proefschrift aangezien deze het meest frequent worden getroffen door de complicaties en geassocieerd zijn met een hoge morbiditeit en mortaliteit.

Samenvatting van de belangrijkste bevindingen

I. De hoeksteen van de behandeling voor patiënten met primair antistof deficiëntie is het gebruik van immunoglobuline (Ig) therapie en / of profylactische antibiotica. In *hoofdstuk 2* worden alle aspecten van Ig therapie bij patiënten met een primaire antistof deficiëntie besproken. Het gunstige effect van Ig therapie op de frequentie en ernst van infecties en de overlevingskansen van CVID patiënten is onbetwistbaar. Echter, Ig therapie lijkt het optreden van de CVID gerelateerde complicaties niet te voorkomen.
II. In *hoofdstuk 3* hebben we het spectrum en de prevalentie van infecties, CVID gerelateerde complicaties en de immunologische kenmerken bij CVID, IgG subklasse deficiëntie- en SADNI beschreven. Recidiverende infecties (voornamelijk luchtwegen) waren in 90% van de CVID patiënten de belangrijkste presentatie en het aantal recidiverende infecties verminderde in 70-100% van deze patiënten na de start van Ig therapie. De ontwikkeling van chronische longziekte en de diverse CVID gerelateerde complicaties ging, ondanks Ig therapie onverminderd door. We hebben een duidelijke relatie vastgesteld tussen specifieke immunologische parameters in de B- en T-lymfocyt compartiment en de specifieke CVID gerelateerde complicaties zoals auto-immuunziekte en granulomateuze ziekte. In vergelijking, IgG subklasse deficiëntie en SADNI patiënten hadden geen complicaties ontwikkeld tijdens de follow-up en we vonden geen afwijkingen in het T-lymfocyt compartiment bij deze patiënten. Uit de literatuur is gebleken dat CVID vaak in een relatief laat ziektestadium wordt opgespoord, terwijl vroegere detectie tot een (veel) betere prognose zou kunnen leiden. We vonden een aanzienlijke 'diagnostic delay' van de diagnose CVID (5-16 jr.). De diagnostic delay' in de groep patiënten met één of meer CVID gerelateerde complicaties was significant langer in vergelijking met de groep patiënten zonder complicaties. Het verhogen van het bewustzijn ('awareness') van de ziekte CVID bij artsen (huisartsen, longartsen, KNO artsen en internisten) zou een positieve bijdrage kunnen leveren aan het klinisch beloop en de prognose van CVID.
III. CVID patiënten hebben frequent aandoeningen van het maagdarm-kanaal. Tevens wordt bij CVID patiënten een relatief hoog risico

op maag- en darm kanker beschreven. Bij XLA patiënten worden ook gevallen van maag- en darmkanker gerapporteerd. De exacte prevalentie van maagdarmkanaal aandoeningen en de bijbehorende symptomatologie is grotendeels onbekend. Evenzo zijn er geen gegevens beschikbaar over de rol van screening bij asymptomatische patiënten. Gezien de veronderstelde risico's en gevolgen van maag- en darmkanker is door sommige onderzoekers routinematige evaluatie van het maagdarmkanaal bij patiënten met CVID en XLA gesuggereerd, echter de frequentie waarmee deze screening verricht zou moeten worden is niet vastgesteld. In *hoofdstuk 4* beschrijven we de prevalentie en het spectrum van maagdarm pathologie vastgesteld door endoscopische screening. Tevens hebben we aan de hand van de bevindingen een voorstel gedaan voor het screenen van CVID en XLA patiënten op maagdarm aandoeningen. We vonden een hoge prevalentie van maagdarm afwijkingen bij CVID patiënten (83%). Bij 30% van deze patiënten hadden de bevindingen therapeutische gevolgen, zoals oesofagitis, Helicobacter pylori geassocieerde gastritis en adenoom van de dikke darm. Bij slechts 44% van CVID patiënten konden symptomen worden gecorreleerd aan endoscopische bevindingen. Bovendien bleek bij 40% van de asymptomatische patiënten weldegelijk maag- en darm afwijkingen aanwezig te zijn, zoals Helicobacter pylori geassocieerde gastritis (maagontsteking), wat een risicofactor is voor maagkanker. De laatste twee bevindingen worden als belangrijke risicofactoren beschouwd voor maag- en darmkanker. Bij XLA patiënten vonden we op relatief jonge leeftijd poliepen in de dikke darm. De maagdarm screening door middel van endoscopie is vooral gericht op de detectie van pre- maligne maag- en darmaandoeningen zoals Helicobacter pylori gastritis en poliepen (adenomen) in de dikke darm. Wij stellen voor om bij volwassen CVID en XLA patiënten ten tijde van het stellen van de diagnose van CVID een gastro-en colonoscopie te verrichten. Het vervolg zal afhankelijk zijn van de ernst en het type van de bevindingen vergelijkbaar met de algemene bevolking. In het geval van normale bevindingen bij de initiële screening, stellen wij voor om de gastroscopie elke 3-5 jaar bij CVID patiënten te herhalen en een colonoscopie om de 5 jaar te herhalen bij CVID en XLA patiënten.

IV. Vanwege de geassocieerde verhoogde morbiditeit en mortaliteit van chronische longziekten bij CVID patiënten is vroegtijdige detectie en monitoren essentieel zodat tijdige behandeling gestart zou kunnen worden. In *hoofdstuk 5* beschrijven we de prevalentie en het spectrum van (preklinische) chronische longziekte bij CVID patiënten met behulp van Computed Tomography (CT) scans en longfunctietesten. Tevens hebben we aan de hand van de bevindingen een voorstel gedaan voor het screenen van CVID patiënten op longafwijkingen. CT scans werden gescoord voor structurele luchtweg afwijkingen en interstitiële longziekte (ILD) met behulp van een gevalideerd scoresysteem. In vrijwel alle CVID patiënten werden ondanks adequate Ig therapie subtiele preklinische pulmonale afwijkingen gedetecteerd. Significant structurele luchtweg afwijkingen (airway score> 7) of interstitiële longafwijkingen (ILD score> 5) waren aanwezig in 30% en 34% van de patiënten. Bronchiectasiëen waren in maar liefst 64% van de patiënten aanwezig en 75% van de patiënten bleken reeds voorstadia van bronchiectasiëen te hebben. Het optreden van lage luchtweginfecties waren voorspellend voor het ontstaan van de structurele luchtweg afwijkingen. Opvallend is dat deze de luchtwegafwijkingen zijn ontstaan ondanks goed ingestelde Ig therapie. Mogelijkerwijs bestaan er andere (nog te onderzoeken) mechanismen, naast recidiverende infecties, waardoor deze afwijkingen kunnen ontstaan. De correlatie tussen de CT- scores en de resultaten van de longfunctietesten in onze studie was slecht. Met betrekking tot de screening voor longaandoeningen zijn herhaalde CT-scans met gestandaardiseerde score systemen betrouwbaar en reproduceerbare instrumenten om progressie van pulmonale afwijkingen te vervolgen. Longfunctietesten blijken uit onze (en andere studies) een slechte voorspeller voor subtiele long afwijkingen. Wij stellen voor om bij volwassen patiënten ten tijde van het stellen van de diagnose van CVID een initiële HRCT scan te verrichten. Het vervolg zou, op basis van onze studie, bestaan uit 2-of 3-jaarlijkse verrichten van een CT scan bij CVID patiënten met:
(I) aanwezigheid van een auto-immuun ziekte;
(II) \ met frequent optreden van lagere luchtweginfecties
(III) met een significante AD score (>7) of ILD score (>5) op de initiële CT-scan.

Voor de CVID patiënten die niet voldoen aan bovenstaande criteria zou de screening met CT scan eenmaal per vijf jaar voldoende kunnen zijn.

V. Vanwege de verhoogde morbiditeit en mortaliteit door long- en maag- en darm complicaties bij CVID patiënten is vroegtijdige detectie en follow up van de dergelijke aandoeningen essentieel. Met als doel patiënten te identificeren met een hoger risico op maag en darm- en long complicaties, zochten wij in *hoofdstuk 6* naar verschillen in klinische variabelen en kenmerken in het B- en T- lymfocyten compartiment tussen CVID patiënten met goed gedefinieerde maagdarm- en pulmonale afwijkingen in vergelijking met CVID patiënten zonder deze complicaties. De belangrijkste bevinding was dat ernstige tekenen van ILD geassocieerd was met de aanwezigheid van auto-immuunziekten en gecorreleerd kon worden aan specifieke T- en B- lymfocyt fenotypes. Structurele luchtwegaandoeningen konden worden gecorreleerd aan het optreden van lage luchtweginfecties maar niet aan specifieke B- en T- lymfocyt fenotypes. Wij identificeerden minder uitgesproken abnormaliteiten in B- en T- lymfocyten compartiment van patiënten met darm afwijkingen. Klinische variabelen zoals leeftijd, CVID-ziekteduur en IgG dalspiegel waren niet verschillend tussen de groepen patiënten met en zonder complicaties.

Conclusie

Ons onderzoek heeft aangetoond dat het klinische spectrum van CVID patiënten divers is en dat de ontwikkeling van niet-infectieuze complicaties een continu proces is ondanks Ig therapie. Het gunstige effect van Ig therapie op de korte-termijn (optreden van acute luchtweginfecties en antibioticagebruik) is onbetwistbaar. Echter, het gunstige effect op de lange termijn complicaties (voorkomen van niet-infectieuze complicaties en eindorgaanschade) lijkt afwezig. De ontwikkeling van deze complicaties is te wijten aan de gecombineerde ontregeling in het B- en T- lymfocyt compartiment. Patiënten met auto-immuun ziekten, lymfoproliferatieve complicaties en interstitiële longaandoeningen bleken inderdaad een specifiek B- en T-lymfocyt fenotype hebben. De gevonden specifieke immunologische fenotypes

zijn kandidaat markers om patiënten te identificeren met een verhoogde kans op deze complicaties. Dit zou kunnen bijdragen aan een verbeterde diagnostiek, follow up en behandeling van de patiënten. Repetitieve screening op maag- en darm aandoeningen en longafwijkingen in longitudinale observationele cohortstudies zullen nodig zijn om de optimale follow-up frequentie vast te stellen. Tot die tijd hebben wij naar aanleiding van onze bevindingen het genoemde screenings voorstel gedaan. Tenslotte, vonden wij een aanzienlijke 'diagnostic delay' bij CVID patiënten, vooral bij patiënten met niet-infectieuze complicaties. Wanneer het ziektebeeld CVID (te) laat wordt herkend zal de start van adequate therapie worden vertraagd, wat in bepaalde gevallen kan leiden tot aanzienlijke morbiditeit. Het is dus belangrijk om door middel van onderwijs en nascholing de kennis over de variabele klinische presentaties en manifestaties van CVID onder artsen te verhogen.

Totdat genetische analyse alle immunologische defecten van CVID heeft ontrafeld, blijft CVID een heterogene populatie van patiënten die klinische kenmerken delen maar ook zeer verschillen in klinisch beloop en de prognose.

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'By all means recognise secretaries, wives or husbands, lovers and parents- but not in the manuscript'. (Spence AA, Discussion. In G M Hall, ed. How to write a paper, BMJ Publishing Group 1994)

Nu mijn proefschrift is afgerond is het moment aangebroken voor het schrijven van het dankwoord. Ondanks het feit dat ik natuurlijk mijn lieve en altijd geïnteresseerde schoonfamilie (*Ria, Leo, Peter en Joleen*), zwager (*Eddy*) vriendinnen en vrienden, boksclub, zangclub en wat dies meer zeer dankbaar ben voor het feit dat ze mijn leven zo mooi kleuren, wil ik me beperken tot de mensen die direct een bijdrage hebben geleverd aan dit proefschrift.
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Liselotte Jacobien Ellerbroek was born on May 1st 1970 in Amsterdam, The Netherlands. After she graduated in 1989 from the 'Rijnlands lyceum' in Oegstgeest, she went to Grenoble to practice French language and literature at the Université Stendhal des Langues et Lettres de Grenoble. In 1990 she started medical school at the University of Amsterdam/ Academic medical centre (AMC). During her studies she performed research during 3 months at the department of surgery at the Free university medical hospital (VUMC) Amsterdam but also enjoyed student live in Amsterdam. In 1998 she obtained her medical degree and started residency in cardiology at Leyenburg Hospital, the Hague (currently named Haga Hospital) and the Free university medical hospital (VUMC) Amsterdam, under supervision of Prof.dr.C.A.Visser (†). In 2002 she decided to switch from the specialisation cardiology to internal medicine and worked at the St Lucas Andreas Hospital/ Academic medical centre (AMC), Amsterdam, under supervision of dr. J.J.M van Meyel and Prof. dr.P.Speelman respectively. In 2006 she started her clinical fellowship infectious diseases at the University Medical Centre of Utrecht (UMCU) under supervision of Prof. dr. A.I.M. Hoepelman. In 2008 she was registered as an internal medicine and infectious disease specialist and started working at the department of internal medicine & infectious diseases. Meanwhile, she started the research described in this thesis. In May 2013 she was also registered as internal medicine/ emergency medicine specialist. Jacobien is married to Remy Maarschalk, and they have a son (Loed, 2006) and a daughter (Wies, 2009).