UNIVERSITA' DEGLI STUDI DI PADOVA

Corso di Laurea Magistrale in Medicina e Chirurgia

Dipartimento di Medicina – DIMED

Direttore: Prof. Paolo Simioni

U.O.C. Medicina Generale I

Ospedale Ca' Foncello di Treviso

TESI DI LAUREA

"Granulomatous Lymphocytic Interstitial Lung Disease in Common Variable Immunodeficiency: evidence from an Italian multicenter retrospective study"

Relatore: Prof. Francesco Cinetto

Correlatrice: Dott.ssa Helena Buso

Laureanda: Stefani Rebecca

Anno Accademico 2023/2024

INDEX

1. Abstract

Introduction. Common Variable Immunodeficiency (CVID) is the most prevalent symptomatic Primary Antibody Deficiency (PAD) of the adult and the most diagnosed symptomatic Inborn Error of Immunity (IEI). Its manifestations can be infectious or non-infectious, mirroring the dichotomy of the disease between immune deficiency and immune dysregulation. Granulomatous Lymphocytic Interstitial Lung Disease (GLILD) is one of CVID's most severe complications, characterized by poorer outcome and almost unknown pathogenesis.

Goals. The aim of the study was to analyse GLILD in terms of mortality, pulmonary function, clinical phenotypes and therapy. Moreover, we tried to identify clinical and immunological parameters associated with a negative prognosis.

Methods. We considered patients with histological and/or clinical-radiological pictures suggestive of GLILD, referred to four Italian centers. Among these, those with characteristics that suggested a more severe condition (*e.g.* lymphomas, need for immunosuppressive therapy, death) were enrolled in the negative prognosis group (cases). The remaining GLILD patients were enrolled in the good prognosis group (controls). Data were collected via retrospective review of medical records of cases and controls.

Results. We enrolled 64 patients in the general cohort, from which we obtained the following results: female sex was predominant (72%); lymphoid follicular hyperplasia and granulomas were the main findings at lung and lymph node biopsy (respectively 77% and 95% for hyperplasia, 69% and 63% for granulomas); 12 patients had available genetic tests, 4 of which showed a mutation of TACI (33.3%) and 3 of CTLA-4 (25%); immunoglobulins were all below normal range (median IgG = 236 mg/dL; IgA = 10 mg/dL; IgM = 18 mg/dL), being the reason why the totality of the cohort was under replacement therapy. Extrapulmonary involvement was highly frequent (85%), with 77% of patients presenting splenomegaly, 31% hepatopathy, 50% Immune

thrombocytopenia, 20% Autoimmune hemolytic anemia. ^{18}FDG-PET-CT scans detected involvement of supradiaphragmatic (97%) and subdiaphragmatic lymph nodes (85%), lung (85%) and spleen (61%). 25% of the patients had cancer, half of which were hematologic. Focusing on lung involvement, DLCO% was reduced at pulmonary function tests (median of 77% at first and 75% at last PFT), while CT scans showed mainly signs of lymphadenopathies (79%), nodules (75%), bronchiectasis (54%) and ground glass opacities (50%). 63% of the cohort underwent specific therapy (steroids, rituximab, DMARDs); out of them, 67.5% were treated with rituximab and 52% of them had clear evidence of response. The mortality rate of the cohort was 17% and the main GLILD-related causes of death were infections (46%), lymphomas (18%) and hepatopathy (9%).

The analysis of the case-control groups led to these results: in cases, female prevalence was higher (82% vs 58%; *p=0.050**), diagnostic delay was longer (9 vs 3 years; *p=0.002***), specific histological findings were more frequent, IgG levels at diagnosis were significantly lower (189 vs 298 mg/dL; *p=0.027**). Then, at laboratory tests, the CD4+/CD8+ ratio was significantly lower in cases than controls, at both serum (1.10 vs 1.58; *p=0.050**) and BALF analysis (1.10 vs 4.13; *p=0.004***). Moreover, lung function parameters were all lower in the cases, with a significant reduction in DLCO% at first PFT (70.5% vs 91.0%; *p=0.002***). Regarding radiological findings, cases had significantly higher percentages of ground glass opacities (71% vs 19%; *p<0.001****) and bronchiectasis (71% vs 6%; *p=0.004***).

Finally, we proposed a prognostic score composed by IgG levels at diagnosis, years of diagnostic delay since CVID diagnosis and DLCO% at first available PFT, obtaining a specificity of 95.2%, a sensitivity of 75% and an area under the curve (AUC) of 0.901.

Conclusion. Our study confirmed some well-known features of GLILD and proposed new matters for reflection. Moreover, we highlighted the different aspects that may distinguish milder conditions from more severe ones, aiming to design a prognostic score.

In any case, GLILD still remains a partially unknown entity and more studies are needed, hopefully prospective and with larger cohorts, since a more standardized and evidence-based approach to the diagnosis and treatment of this condition is needed.

1.1 Abstract - Italiano

Introduzione. L'Immunodeficienza Comune Variabile (CVID) è l'Immunodeficienza Anticorpale Primitiva (PAD) più diffusa nell'adulto e l'*Inborn Error of Immunity* (IEI) sintomatico più diagnosticato. Le sue manifestazioni possono essere infettive e non, riflettendo la dicotomia della patologia, divisa fra infezione e autoimmunità. La Malattia Polmonare Interstiziale Granulomatosa Linfocitica (GLILD) è una delle più severe complicanze della CVID, caratterizzata da prognosi sfavorevole e patogenesi quasi completamente sconosciuta.

Obiettivi. L'obiettivo dello studio era analizzare la GLILD in termini di mortalità, funzionalità polmonare, fenotipi clinici e terapia. Inoltre, abbiamo cercato di identificare i parametri clinici e immunologici associati ad una prognosi negativa.

Metodi. Abbiamo considerato pazienti con quadri istologici o clinico-radiologici suggestivi di GLILD, seguiti in quattro centri di riferimento italiani. Tra di essi, coloro che presentavano caratteristiche indicative di una condizione più severa (*e.g.* linfoma, necessità di terapia immunosoppressiva, decesso) sono stati inclusi nel gruppo a prognosi negativa (casi). I pazienti rimanenti sono stati inclusi nel gruppo a prognosi favorevole (controlli). I dati sono stati raccolti tramite una revisione retrospettiva delle cartelle cliniche dei casi e dei controlli.

Risultati. Abbiamo arruolato 64 pazienti nella coorte principale, dalla quale abbiamo ottenuto i seguenti risultati: vi era una netta prevalenza del sesso femminile (72%); l'iperplasia follicolare linfoide ed i granulomi erano i reperti più presenti alle biopsie di polmone e linfonodo (rispettivamente 77% e 95% per quanto riguarda l'iperplasia, 69% e 63% per i granulomi); 12 pazienti avevano svolto test genetici, 4 dei quali hanno mostrato mutazioni del gene TACI (33.3%) e 3 di CTLA-4 (25%). I livelli di tutte le classi di immunoglobuline erano inferiori alla norma (mediana di IgG = 236 mg/dL; IgA = 10 mg/dL; IgM = 18 mg/dL); per questo motivo l'intera coorte era sottoposta a terapia sostitutiva. L'interessamento extra-polmonare era largamente frequente (85%), con 77% dei

pazienti presentanti splenomegalia, 31% epatopatia, 50% Trombocitopenia autoimmune, 20% Anemia emolitica autoimmune. Alla ^{18}FDG-PET-TC è stato riscontrato l'interessamento dei linfonodi sovradiaframmatici (97%) e sottodiaframmatici (85%), dei polmoni (85%) e della milza (61%). Il 25% dei pazienti era affetto da neoplasie, metà delle quali ematologiche. Per quanto riguarda l'interessamento polmonare, la DLCO% era diminuita alle spirometrie (con una mediana di 77% del predetto alla prima spirometria e 75% all'ultima), mentre le TC erano caratterizzate principalmente da linfadenopatie (79%), nodularità (75%), bronchiectasie (54%) e opacità a vetro smerigliato (50%). Il 63% della coorte è stato sottoposto a terapia specifica (steroidi, rituximab, DMARDs); di questi, 67.5% sono stati trattati con rituximab ed il 52% di essi ha dimostrato chiara evidenza di risposta. La mortalità della coorte è risultata essere del 17% e le principali cause di morte correlate a GLILD sono state le infezioni (46%), i linfomi (18%) e l'epatopatia (9%).

Il successivo confronto tra casi e controlli ha portato a questi risultati: nel gruppo dei casi, il sesso femminile era presente in percentuale ampiamente maggiore nei casi (82% vs 58%; *p=0.050**), il ritardo diagnostico era più protratto (9 vs 3 anni; *p=0.002***), i reperti istologici tipici erano più frequenti, i livelli di IgG alla diagnosi erano significativamente più bassi (189 vs 298 mg/dL; *p=0.027**). Inoltre, agli esami bio-umorali, il rapporto CD4+/CD8+ era significativamente ridotto nel gruppo dei casi rispetto ai controlli, sia a livello sierico (1.10 vs 1.58; *p=0.050**) che al livello del liquido di lavaggio broncoalveolare (1.10 vs 4.13; *p=0.004***). Oltre a ciò, i parametri di funzionalità polmonare erano tutti peggiori nel gruppo dei casi, con una riduzione significativa della DLCO% alla prima spirometria disponibile (70.5% vs 91.0% del predetto; *p=0.002***). Quasi la totalità dei reperti radiologici tipici era maggiormente frequente nei casi rispetto ai controlli, con differenza significativa per quanto riguardava le opacità a vetro smerigliato (71% vs 19%; *p<0.001**** e le bronchiectasie (71% vs 6%; *p=0.004***).

Abbiamo, infine, elaborato una proposta di score prognostico che tiene conto dei livelli di IgG alla diagnosi, del ritardo diagnostico (relativo alla diagnosi di CVID) e della DLCO (espressa in percentuale del predetto) alla prima spirometria

disponibile, ottenendo una specificità del 95.2%, una sensibilità del 75% ed un'area sotto la curva (AUC) di 0.901.

Conclusioni. Il nostro studio ha confermato alcune caratteristiche già note della GLILD e ha proposto qualche nuovo spunto di riflessione. Inoltre, abbiamo evidenziato degli aspetti che potrebbero aiutare a distinguere sin dall'inizio le condizioni più benigne da quelle a prognosi più severa, con l'intento di proporre uno score prognostico.

In ogni caso, la GLILD rimane ancora oggi una patologia parzialmente sconosciuta ed ulteriori studi saranno necessari, auspicabilmente prospettici e coinvolgendo coorti più ampie di pazienti. Quel che è certo è che è necessario un approccio diagnostico e terapeutico più standardizzato e basato sull'evidenza per affrontare in modo ottimale questa condizione.

2. Introduction

2.1 Inborn Errors of Immunity (IEIs)

Inborn errors of immunity (IEIs) are a heterogeneous group of almost 500 disorders, most of which are defined by a specific underlying gene defect. Previously referred as Primary Immunodeficiency Disorders (PIDs), their name has now changed to highlight the immune dysregulation typical of these conditions.

In the previous classification the defects were divided into two subgroups, according to their belonging to innate and/or adaptive immune response. As a consequence, the classification of these conditions differentiated between "broad spectrum" PIDs and selective PIDs; furthermore, every single gene defect was analysed, to have a more precise view on the matter[1] (*See figure 1*).

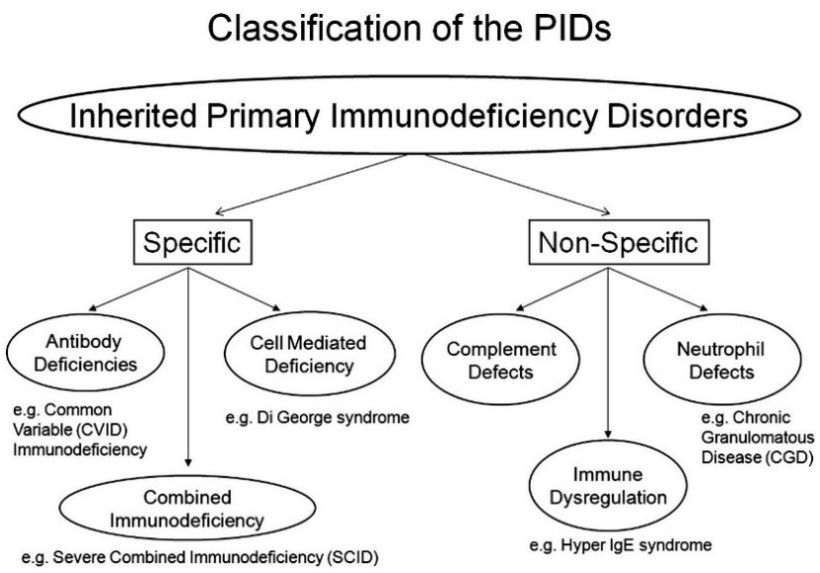


Figure 1:2017 IUIS classification of Inherited Primary Immunodeficiency Disorders (PIDs)[1].

The new classification, starting from the new definition of Inborn Errors of Immunity (IEIs)[2], focuses not only on the immune defect, but also on the dysregulation that affects the immune system of these patients. Moreover, there are multiple subgroups, mirroring its profound heterogeneity (*See Figure 2*).

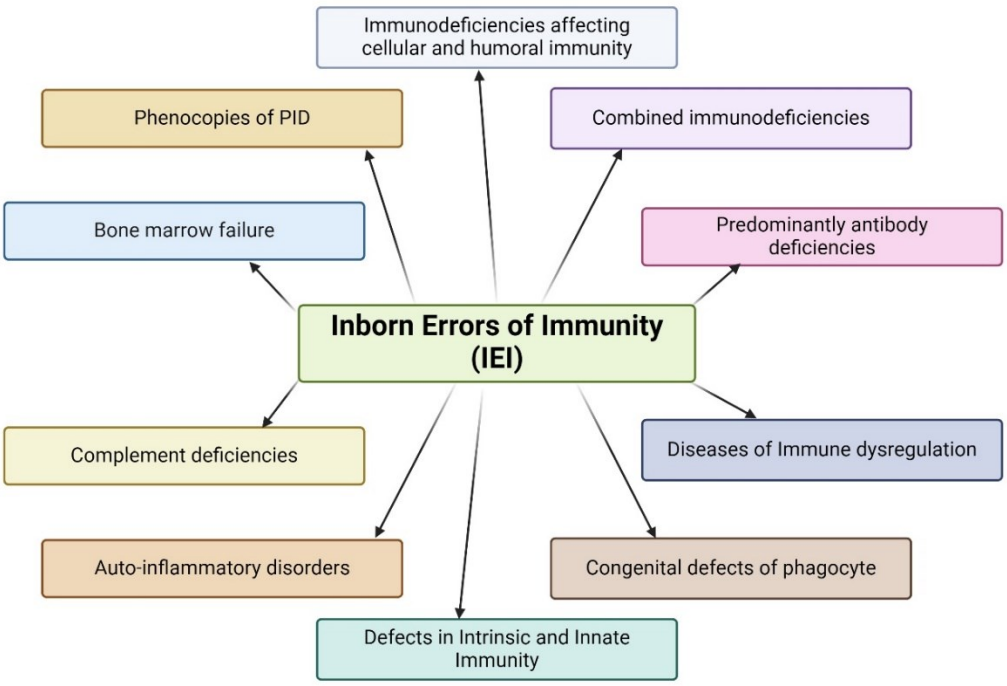


Figure 2: The 2022 Update of IUIS Phenotypical Classification for Human Inborn Errors of Immunity (IEIs). Created with BioRender.com.

If considered individually, IEIs are rare diseases, but their cumulative incidence is significant[3]. The clinical phenotypes range from milder infections to severe manifestations of autoimmunity, auto-inflammation or cancer. This last clinical feature, in particular, represents a major cause of death in patients with Common Variable Immunodeficiency (CVID)[4].

2.2 Primary Antibody Immunodeficiencies (PADs)

Primary antibody deficiencies (PADs) are a vast subgroup of IEIs which include a wide range of diseases. This collective goes from heavy and generalized defects, like X-linked agammaglobulinemia (XLA) - in which there is an intrinsic defect of B cells maturation and a consequential gammaglobulins defect involving all Ig subtypes -, to very specific antibody malfunctions, as Selective antibody deficiency (SAD) – in which there is only a qualitative deficit in response to polysaccharide antigens, without any hypogammaglobulinemia -.

PADs are associated with a better long-term prognosis and a generally adult diagnosis, thus being the most diagnosed IEIs. The defect leading to these conditions can be B-cell intrinsic or extrinsic, but the aetiology is not completely understood. Even T-cell differentiation can be compromised, as T cells are deeply involved in B cell activation[5].

The main components of this group include: the already mentioned XLA and SAD, Hyper-IgM syndromes (which are mainly due to a defective interaction between CD40 and CD40L or defects in isotypic switch-related genes and are characterised by low IgA and IgG but normal or high IgM), Selective IgA deficiency or SIgAD (mainly asymptomatic and characterised by low IgA and normal IgG and IgM), IgG subclass deficiency (in which only one or more IgG subclasses are reduced), unclassified antibody deficiencies (UAD) and Common Variable Immunodeficiency.

2.3 Common Variable Immunodeficiency (CVID)

Common Variable Immunodeficiency (CVID) is the most prevalent symptomatic PAD of the adult[6] and the most diagnosed symptomatic IEI. It cannot be defined as a single disease, but rather as a heterogeneous group of disorders characterized by hypogammaglobulinemia. In a big percentage of the cases, the underlying genetic defects are still unknown; however, nowadays, more and more often these defects are being identified, favouring a more tailored management.

Its previous definition, announced in the International Consensus Document on CVID (ICON), included low serum levels of IgG and IgA and/or IgM in patients aged >4 years showing poor response to vaccination and/or low switched-memory B-cells, after ruling out any other cause of secondary hypogammaglobulinemia[6].

More recently, the European Society for Immunodeficiencies (ESID) agreed that the diagnosis of CVID requires[7]:

At least one of the following:

- Increased susceptibility to infections
- Autoimmune manifestations
- Granulomatous disease
- Unexplained polyclonal lymphoproliferation
- Affected family member with antibody deficiency

AND marked decrease of IgG and marked decrease of IgA with or without low IgM levels (measured at least twice; <2SD of the normal levels for their age)

AND at least one of the following:

- Poor antibody response to vaccines (and/or absent isohemagglutinins)
- Low switched memory B cells (<70% of age-related normal value)

AND secondary causes of hypogammaglobulinemia have been excluded

AND diagnosis is established after the 4th year of life (but symptoms may be present before)

AND no evidence of profound T-cell deficiency, defined as 2 out of the following (y=years of life):

- CD4 numbers/microliter: 2-6y <300, 6-12y <250, >12y <200
- % naive of CD4: 2-6y <25%, 6-16y <20%, >16y <10%
- T cell proliferation absent

The overall prevalence of this condition is set to be between 1 in 25,000 and 50,000 [8].

2.3.1 Clinical manifestations

The main features of CVID include recurrent bacterial infections (mainly involving upper and lower respiratory tract), often in association with autoimmunity, gastrointestinal involvement, splenomegaly, lymphoproliferative disorders and granulomatous organ infiltration. These symptoms can develop at an early age or during adulthood and the clinical phenotype is variable, so the diagnosis can be challenging, and the diagnostic delay can be significant[9]. It has been suggested that CVID patients may be divided into different groups based on the clinical phenotypic they present, paving the way for a more clinical-oriented handling (*See Table I and Figure 5*)[10].

Clinical manifestations of CVID	
Infectious manifestations	***Non-infectious manifestations***
Pneumonia (atypical and typical organisms)	Autoimmune haemolytic anemia
Airways disease	Granulomatous disease (pulmonary or systemic)
Otitis media	Cytopenias
Sinusitis	Splenomegaly
Conjunctivitis	Enteropathy
Enteritis	Polyarthritis
	Interstitial lung disease /GLILD
	Malignancy (lymphoma/MALToma)

Table I: Clinical manifestations of CVID[11].

2.3.2 Classifications

The two main classifications of CVID are the Chapel classification and the Euroclass classification.

2.3.2.1 The Chapel phenotypic classification

The Chapel classification divides CVID patients into different categories according to their clinical phenotype[10 12]. It has been proposed in order to obtain a systematic division between the groups, to search for an eventual underlying common pathogenesis and to standardize the patients' management.

According to the last revision, the categories are:

- No other disease-related complications (described as "infections only" phenotype);
- Cytopenias (thrombocytopenia, autoimmune hemolytic anemia, neutropenia);
- Polyclonal lymphoproliferation (including persistent unexplained lymphadenopathy, lymphoid interstitial pneumonitis, noninfective granuloma);
- Unexplained persistent enteropathy.

It has been reported that different groups have significantly different prognosis. Moreover, it has been described that in 95% of the cases it is possible to make a reliable estimation of the prognosis starting from 5 years since the diagnosis[12].

2.3.2.2 The Euroclass classification

The Euroclass classification analyses the link between flow-cytometric B-cell phenotyping and clinical manifestations (*see Figure 3*) [13]. It gets inspiration from the previous Freiburg classification[14] and Paris classification[15], improving the two methods.

Firstly, it separates patients with nearly absent B cells (less than 1%), identified as group B-, from a B+ group with a higher percentage of B cells. Then, the B+ group is divided according to the number of switched memory B cells: the subgroup with severely reduced smB cells (less than 2%) is called group smB-, while the other is defined as smB+. Thirdly, the smB- group is splat again in accordance with the level of transitional B cells, if more than 9% (Tr^{hi}) or less

(Tr^{norm}). Lastly, the smB+ and smB- groups are also classified in accord with the $CD21^{low}$ B cells count (more or less than 10%).

The applications of this classification are several: for example, the B- group contains all patients with severe defects of early B-cell differentiation, while severely reduced switched memory B cells indicate a defective germinal center development. Moreover, correlations between subgroups and clinical manifestations have been searched: a reduction of smB may be associated with a higher risk for splenomegaly and granulomatous disease, whilst an elevation of $CD21^{low}$ B cells has seemed to be associated with splenomegaly and autoimmune disease; additionally, lymphadenopathy seems to be significantly linked with transitional B-cell expansion.

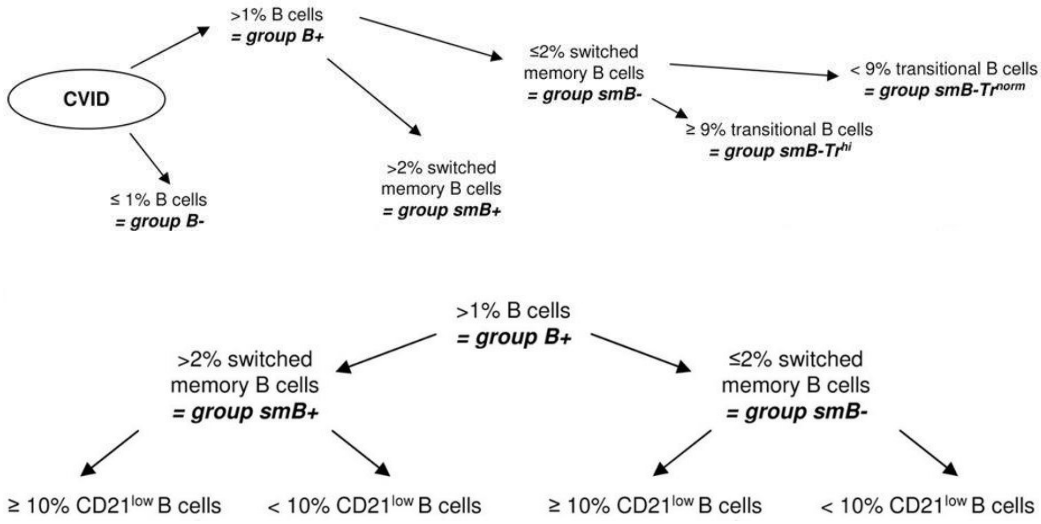


Figure 3: The Euroclass classification scheme[13].

2.3.3 Management

General management of CVID relies upon IgG replacement therapy (IgRT), either using Intravenous Ig (IVIG) or Subcutaneous Ig (SCIG).[16] Both IVIG and SCIG have been proven to be effective in infection prevention, to be safe in terms of infusion related adverse events[17] [18] and to result in improved quality of life for affected patients. Regarding the dysregulatory aspect of CVID, several

immunosuppressive therapies are used to keep the condition under control. Strategies can range from steroids to target therapy, based on the eventual underlying genic defect.

2.4 Pulmonary involvement in CVID

Respiratory disease is an important cause of morbidity and mortality amongst affected patients. The complications include respiratory tract infections (RTIs), airways diseases, malignancy and interstitial lung disease (ILD).

To understand the huge impact of pulmonary involvement in CVID, it is important to remember that RTIs are part of the 10-item-list of warning signs elaborated by Jeffrey Modell Foundation for the diagnosis of PID (*See Figure 4*)[19].

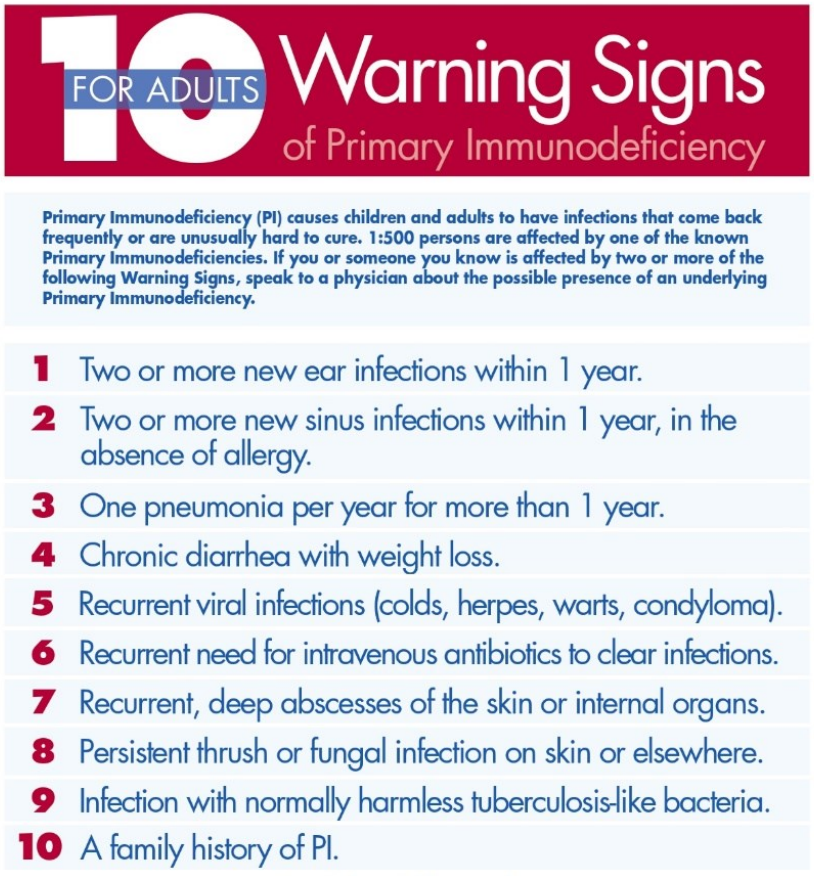


Figure 4: Jeffrey Modell Foundation's 10 Warning signs of Primary Immunodeficiency[19]

Early diagnosis is crucial; pulmonary lung function tests (PFTs) and computed tomography (CT) play an important role in the initial work-up and follow up of CVID patients. The main goal is to detect, characterize, and quantify the extent of lung damage, giving a clear vision of the condition, to direct the treatment.

Pulmonary complications, as other CVID complications, can be schematized into two different categories (*See Figure 5*) [20]:

- Infection-related (*e.g.* pneumonia or "degenerative patterns" as bronchiectasis or early COPD)
- Immune mediated Interstitial Lung Diseases (*e.g.* GLILD)

It is also known that some conditions can be a result of both[21].

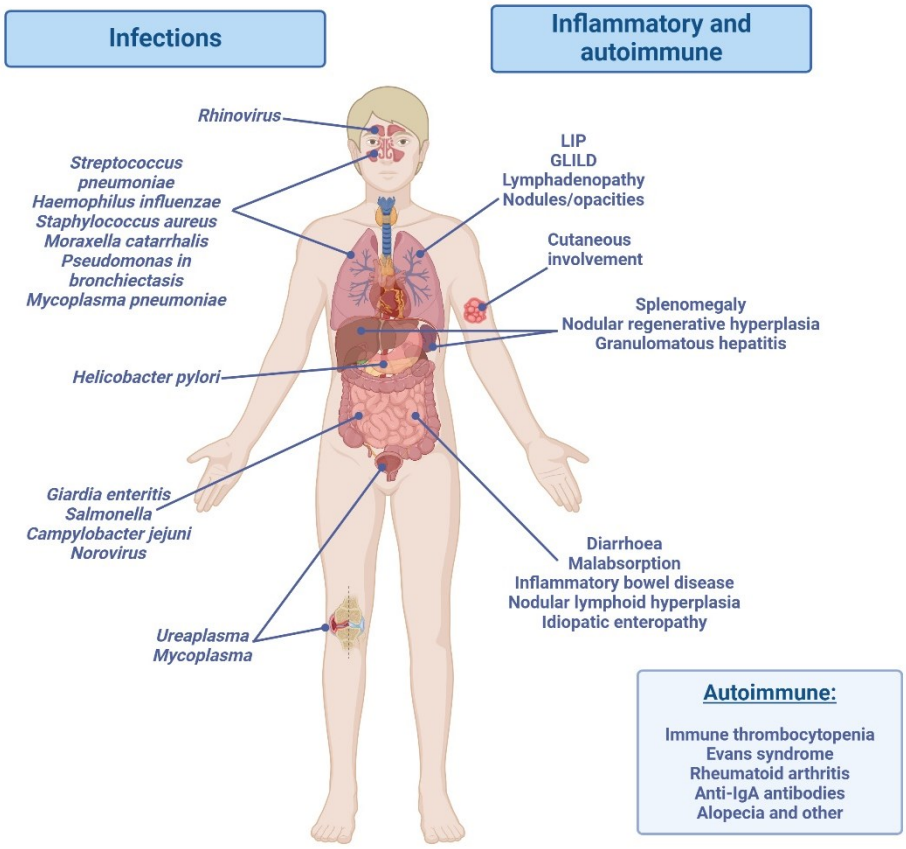


Figure 5: Infectious and non-infectious complications of common variable immunodeficiency[22]*.*
Created with BioRender.com.

2.4.1 Respiratory tract infections (RTIs)

One of the most dramatic aspects of CVID in the past was represented by recurrent lower respiratory tract infections (LRTI) as pneumonia. The majority of the infections in these patients was burdened by high morbidity and mortality. At the current time, thanks to the advances in availability and personalization of the immunoglobulin replacement therapy, management of these conditions has come to the point that they are no more the main source of concern.

As displayed in the graph below (*see Figure 6*), the most common respiratory pathogens are encapsulated pyogenic bacteria, such as *Haemophilus influenzae* and *Streptococcus pneumoniae*. This is the direct result of the suppressed capacity of producing antigen-specific IgG antibodies[23]. The imaging features of bacterial pneumonia mainly revolve around foci of consolidation, lobar or segmental, and bronchiolitis, manifesting as poorly defined centrilobular nodules and tree-in-bud opacities. Cavitations are also possible.

It has also been registered an increased susceptibility to respiratory tract viral infections[24]. Rhinovirus is the main agent, with frequent and prolonged infections, but also Coronavirus and Adenovirus may be present.

Opportunistic pathogens such as *Pneumocystis jirovecii* and *Cytomegalovirus* are less frequent but can be observed in CVID patients with low levels of $CD4^+$ T cells[25].

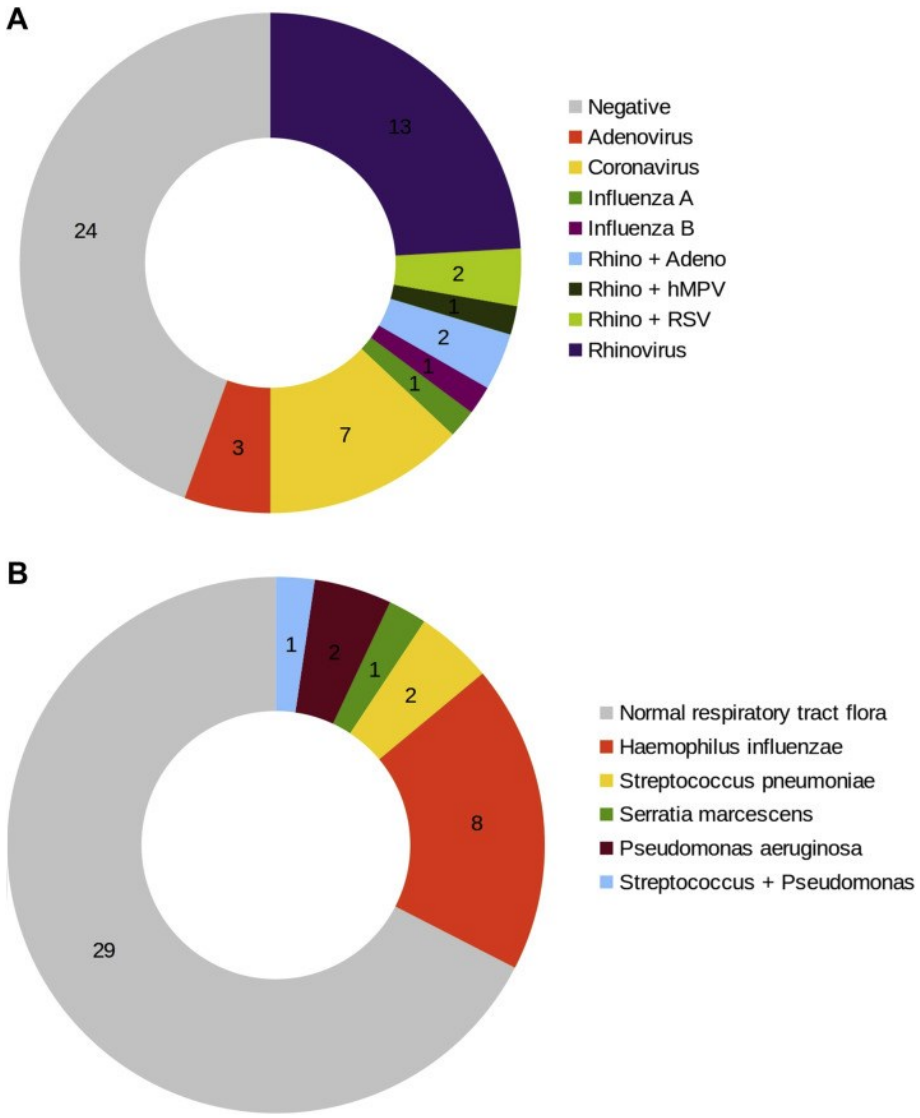


Figure 6: Pathogenic viruses (A) and bacteria (B) analysis from a sample of 69 patients with CVID[26]. Viral PCR was performed on nasopharyngeal swabs in 54 symptomatic patients (A), while bacterial culture was performed on spontaneously expectorated sputum in 43 patients (B).

2.4.2 Infection-related degenerative patterns

Analysing the infection-related side, the recurrence of acute respiratory infections (such as pneumonia) can lead to the development of chronic degenerative diseases (i.e. bronchiectasis and COPD). Indeed, in CVID patients there is a higher risk of suffering from bacterial or viral infections with a slower response to the damage, resulting in delayed or incomplete healing[27]. Furthermore, the need of antibiotic prophylaxis or multiple and aggressive antibiotic treatments, can cause a rise in antibiotic resistance. On the other side, it is not rare to witness

a relevant delay in starting the antibiotic therapy in CVID patients on regular antibiotic prophylaxis, resulting in more severe respiratory exacerbations.[28]

Bronchiectasis and COPD are the main infection-related chronic manifestations in CVIDs patients, being the leading causes of lung function decline[29].

Bronchiectasis is characterised by airways dilatation, resulting in higher risk of developing repeated episodes of infection and inflammation, which worsen the airways' wall and leads to degenerative lung damage. This "vicious cycle" is enhanced in people with CVID, due to the immune dysregulation (*see Figure 7).*

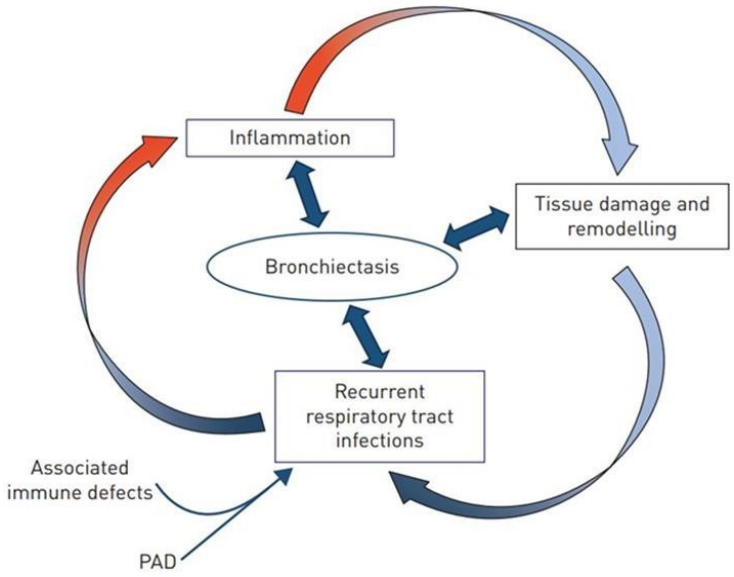


Figure 7: Pathogenesis of bronchiectasis and chronic obstructive lung disease in common variable immunodeficiency (CVID)[20].

As aforementioned, the development of COPD is consequent to the persistence of active inflammatory response to the recurrent infections. Airway hyper reactivity and remodelling are distinctive characteristics of chronic-affected lungs in PADs patients, and these components are known to be causes of COPD in people without smoke or professional exposure or who do not have an α_1-proteinase inhibitor deficiency[30]. Conversely, it has been reported that a significant percentage of frequently exacerbating COPD patients is affected by an underlying PAD,[31] meaning that the connection between the two diseases can be stronger than we actually know.

2.4.3 Bronchiectasis/airway disease

Airways are deeply involved in CVID, and generally this distress is manifested as bronchiectasis, mucoid impaction, and wall thickening. In particular, it has been observed that bronchiectasis and wall thickening are among the most common radiologic pulmonary abnormalities[32 33 34].

Bronchiectasis can affect more than half the CVID population[35], mainly involving the right middle and lower lobes[36]. Likely, this is deeply connected to recurrent lover respiratory tract infections (RTIs)[37], to the point that the severity of the bronchiectasis could be compared to the number of prior RTIs. This may be the reason why patients presenting bronchiectasis are older or have longer treatment delay than patients without them[38].

Typical findings of distal mucoid involvement, resulting from repeated infections, on computed tomography (CT) are parenchymal centrilobular and tree-in-bud nodularity[39]. Moreover, small airway disease may manifest as mosaic attenuation and expiratory air trapping (*see Figure 8*). Visual air trapping and airway thickening are usually associated with obstructive pattern in pulmonary function tests.

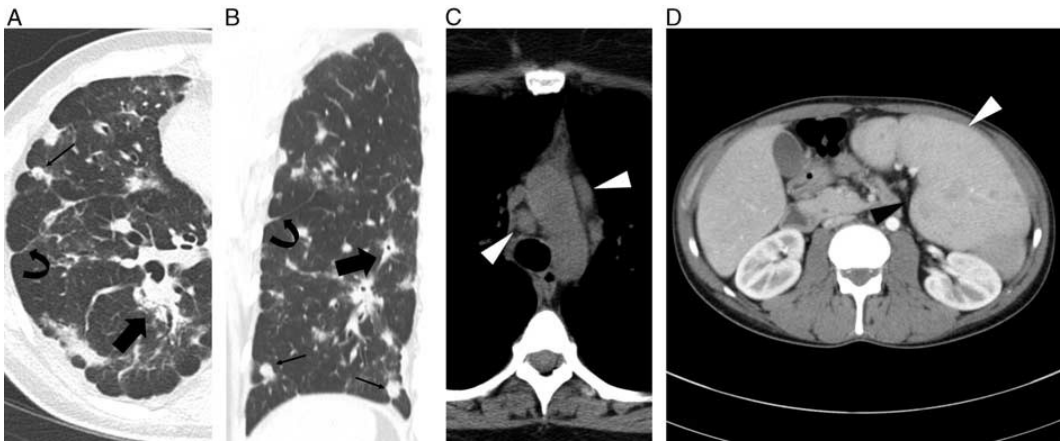


Figure 8: 34-year-old man with CVID and GLILD. A and B, Lung windows show septal thickening (curved arrows), nodularity (thin arrows), and consolidative opacities (block arrows) in a peribronchovascular distribution. Axial soft tissue windows show mediastinal lymphadenopathy (arrowheads) (C) and splenomegaly (arrowheads) (D)[40].

Airway disease is found in almost all CVID patients, highlighting its essential role in the development of the disease. In addition, it may progress even if the patient is under IVIG therapy, contributing to the lung function decrease[41].

2.4.4 Malignancy

Malignancies' incidence in CVID revolves around 2,5-16%, with the main character being non-Hodgkin lymphoma[42]. This condition may emerge from underlying non-clonal lymphoproliferative diseases, with an overall rate quite higher than the general population. Progression occurs despite IVIG therapy[43].

Concerning lung involvement, pulmonary lymphoma is characterized by mediastinal, hilar, and axillary lymphadenopathy on CT, with pulmonary nodules and masses in a perilymphatic distribution (*see Figure 9*) [42].

Mucosa-associated lymphoid tissue (MALT) lymphoma frequently affects the lung, and it may be associated with several immune-mediated diseases (including CVID, but also Sjogren syndrome, SLE, rheumatoid arthritis). CT findings of pulmonary MALT lymphoma show to have mainly a perilymphatic distribution, with subpleural nodularity (solid and/or ground glass) and peribronchovascular extension.

As it can be expected, lymphomas in CVID patients are associated with a poorer overall prognosis than not-oncologic CVID patients[10]. Surprisingly, lung cancer is less frequent in CVID than in the normative population[44].

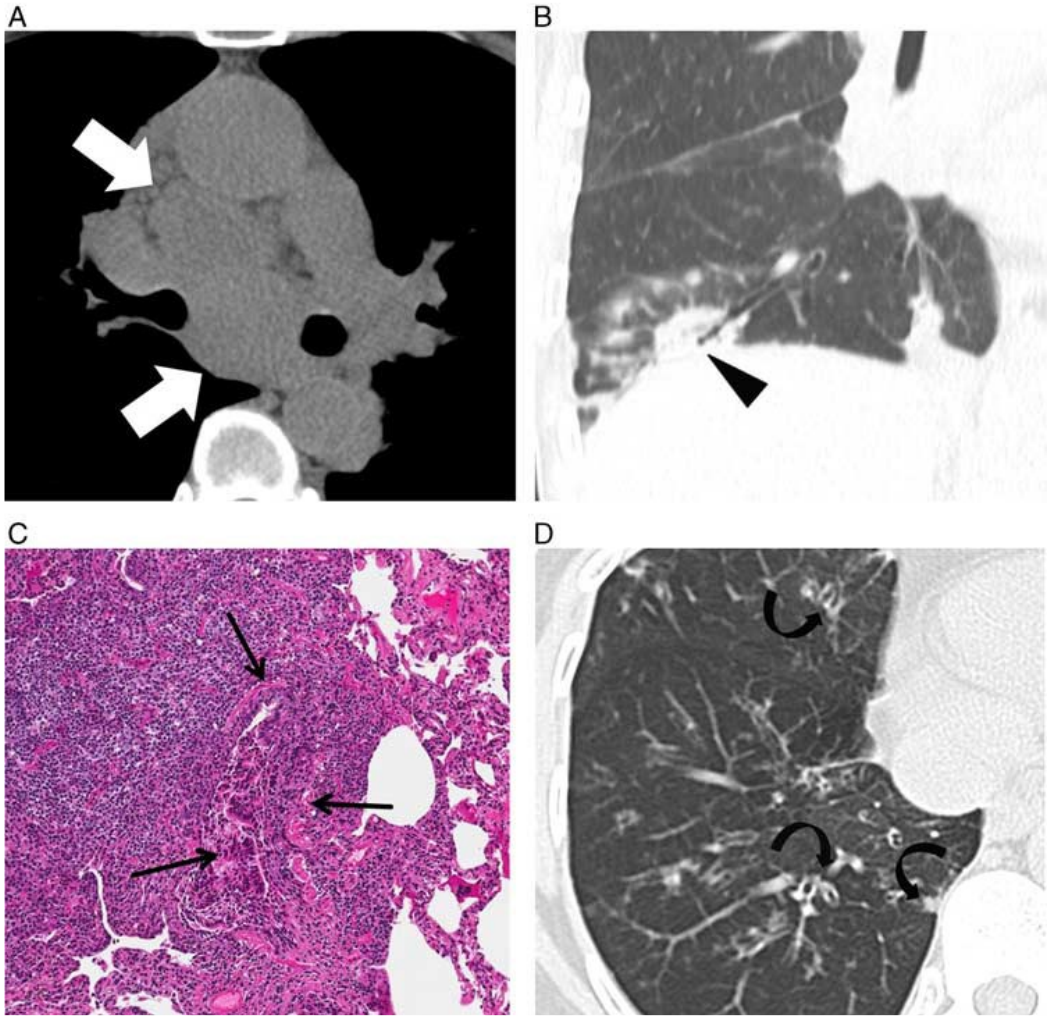


Figure 9: Lymphoproliferative disease in CVID. A, Axial CT in soft tissue windows shows extensive conglomerate mediastinal lymphadenopathy (block arrows). B, Coronal image of lung windows shows multiple multifocal pulmonary masses with air bronchograms (arrowheads) and surrounding satellite nodularity. C, Hematoxylin and eosin stain of a patient with pulmonary MALT lymphoma shows lymphocytic infiltration, effacing normal architecture and destroying the adjacent bronchiole (thin arrows). D, Axial CT image of a patient with CVID complicated by MALT lymphoma shows peribronchovascular thickening and nodularity (curved arrows)[42].

2.4.5 Immune mediated Interstitial lung disease (ILD)

Immune dysregulation is particularly significant in CVID, compared to other PADs, and it is mainly manifested through ILDs. Therefore, Interstitial Lung Diseases (ILDs) have a key role in the clinical development of CVID, to the point that they may be the *main* cause of lung function decline in affected patients[5].

ILDs in CVID patients are cryptic and usually hard to diagnose. The main reason for it is the delayed clinical manifestation, typical of these conditions. Usually,

the symptoms appear when the lungs of the patients are already compromised by fibrosis and the comorbidities (*e.g.* pulmonary hypertension, cor pulmonale, progressive respiratory failure) have already appeared. To intercept the disease at its early stage it can be useful to perform a pulmonary function test (PFT), which can highlight a restrictive pattern and detect any sign of a decreasing diffusion capacity of the lung for carbon monoxide (DLCO). These two parameters can address the patient to second level exams, such as 6-min walk test or high-resolution computed tomography (HRCT), which can expose the underlying disease[45].

There are many ways in which ILDs can be expressed in PADs patients: from follicular bronchiolitis to nodular lymphoid hyperplasia, lymphocytic interstitial pneumonia (LIP), non-specific interstitial pneumonia (NSIP), organising pneumonia and granulomatous lung disease.

Therapy has been very successful at reducing morbidity and mortality coming from infectious causes. As long as infections' prevalence diminishes, non-infectious complications supersede them and set themselves as the main focus of the discussion.

In a subgroup of patients, interstitial lung disease can even be the first manifestation of CVID[46], so it is essential to intercept the disease at its beginning.

There are two main components that characterize interstitial lung disease in CVID: the granulomatous pattern and the lymphocytic pattern. The two of them can co-exist[16], making the diagnosis more difficult. Histologically, the granulomas that can be seen in CVID patients are non-necrotizing, with various composition, either clearly delimited or with blurred borders[46]. Again, biopsies can recognize an overlap of findings, mainly attributable to lymphocytic interstitial pneumonia (LIP), organizing pneumonia (OP) and granulomatous disease.

It is essential to understand that, while the most common organ of involvement is the lung, this is a systemic disease, as granulomas can be found in several other sites.

This thesis is intended to present the main features of the last manifestation aforementioned: in particular, the objective of the dissertation is to analyse the impact and the management of Granulomatous lung disease in Common Variable Immunodeficiency (CVID) patients.

2.5 Granulomatous Interstitial Lung Disease (GLILD)

2.5.1 Definition

Granulomatous Lymphocytic Interstitial Lung Disease (GLILD) is a rare complication of CVID. It has been defined as "a distinct clinic-radio-pathological ILD occurring in patients with [common variable immunodeficiency disorders], associated with a lymphocytic infiltrate and/or granuloma in the lung, and in whom other conditions have been considered and where possible excluded"[47].

GLILD is a condition mainly affecting the lungs of CVID patients, but it may have a wider spectrum of distribution: besides lungs, the main sites involved appear to be lymph nodes, liver and spleen, leading to lymphadenopathy, hepatomegaly and splenomegaly.

It is crucial to intercept the disease before it is too spread out. Additionally, early diagnosis is fundamental because it has been observed that in some cases GLILD appears as the first manifestation of CVID[48].

2.5.2 Incidence and prognosis

GLILD is a very rare condition in the general population, but it is a significant complication in the CVID subgroup. It has been estimated that GLILD can be diagnosed in around 20% of CVID cases[47].

As it concerns prognosis, GLILD is burdened by a poorer outcome than CVID. When observing the Kaplan-Meier survival plot below (*see Figure 10*), it is possible to note that the median survival in Groups 1, 2 and 3B, representing CVID non-GLILD patients, is significantly higher than the median survival of Group 3A, composed of GLILD patients (precisely, 28.8 to 13.7)[49].

Moreover, in 3A group GLILD is associated with dyspnoea, splenomegaly, restrictive pulmonary physiology and consolidation, ground-glass or reticular radiographic abnormalities. These complications may have a key role in worsening the prognosis and the quality of life of these patients.

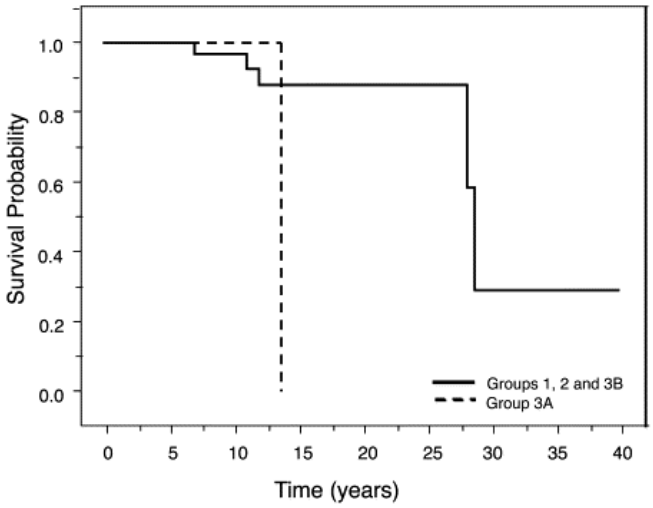


Figure 10: Kaplan-Meier survival plot demonstrating differences between the 2 groups of patients (GLILD vs non-GLILD). The median survival of 28.8 years in groups 1, 2, and 3B (solid line) *is compared with the median survival of 13.7 years in group 3A* (dashed line; $P < .001$). *There is no statistical difference in survival between groups 1, 2, and 3B. Time is from the date of CVID diagnosis*[49].

2.5.3 Pathogenesis

GLILD pathogenesis is not completely understood yet. However, there have been several studies analysing if there may be some facilitating or promoting factors.

Firstly, it has been highlighted the role of germline CTLA-4 haploinsufficiency in favouring lymphoproliferation, lymphocytic infiltration of non-lymphoid organs, autoimmune cytopenias and B cells abnormalities, with an accumulation of $CD21^{low}$ B cells[50]. Cytotoxic T lymphocyte antigen-4 (CTLA-4) is an inhibitory receptor constitutively expressed by $FoxP3^{+}$ T_{reg} cells, which are deeply involved in the maintenance of tolerance. Moreover, it is expressed by activated T cells, with a suppressive function. Actually, CTLA-4 has been heavily exploited to modulate immunity in autoimmune and cancer patients, thanks to CTLA4-Ig fusion proteins and neutralizing CTLA-4 antibodies. When mutated, CTLA-4 expression can decrease significantly, contributing to a severe loss of tolerance and infiltrative auto-immune disease.

T cells are deeply interested in GLILD: raised serum markers of T cell activation and exhaustion have been detected (*e.g.* sCD25, sTIM-3, IFN-γ, TNF)[51],

suggesting that these cells may have a key role in the pathogenesis of this condition.

On the other side, it is *not* just the T cell phenotype that has been detected as altered in GLILD patients.

It has already been studied that most of CVID patients, regardless of GLILD status, have higher frequency of $HLADR^{+}CD4^{+}$ T cells, $CD57^{+}CD8^{+}$ T cells, and $CD21^{low}$ B cells when compared to healthy controls. However, *only* in CVID/GLILD patients it has been observed an alteration of TCR/BCR signalling in the activated lymphocyte populations[52].

Therefore, B cell dysregulation is also an essential component in GLILD pathogenic pathway. For example, an increasing B cell activating factor (BAFF) can be included in the potential mechanisms of disease of GLILD[53]. BAFF is a cytokine upregulated by the interferon gamma (IFN-γ) and a component of the STAT1 signalling axis, which heavily influences B cell activation and survival. BAFF is also a member of the TNF ligand superfamily and it is produced mainly by monocytes, macrophages, neutrophils and dendritic cells. This cytokine binds three receptors on the surface of B cells: the BAFF receptor (BAFFR), calcium modulator and cyclophilin ligand interactor (TACI) and B cell maturation antigen (BCMA)[54]. These receptors are vital to the activation, proliferation and survival of B cells. BAFFR, for example, is involved in the survival and maturation of peripheral B cells, while TACI can inhibit B cell expansion, induce IgG and IgA class switch recombination and promote plasma cells differentiation ad survival. An elevation of B cell-activating factor (BAFF) has been found both in serum and in lungs of CVID patients with ILD[55]. Moreover, lymphoid hyperplasia in CVID-ILD has been analysed to be a consequence of a BAFF driven B cells stimulation (*see Figure 11*)[55]. Lastly, according to recent studies, TACI signalling may be involved in the immune dysregulation cascade and may be correlated to the development of some autoimmune disorders (*e.g.* SLE)[56 57].

Another risk factor which may be included is high IgM serum levels: it seems that this might correlate with a higher activity of pulmonary B cells, leading to increased follicle formation and sped up ILD development[55].

So far, it has been discussed the role of humoral and cell-mediated adaptive immunity. Nevertheless, innate immunity is also essential in the pathogenesis of GLILD. One of the main histopathological features of GLILD is the sarcoid-like non-caseating granuloma, composed of giant cells and epithelioid histiocytes. These cells descend from the monocyte-macrophage lineage through the mTORC1 pathway, which many studies have suggested as the main driver of the granuloma formation in sarcoidosis[58]. The mechanism seems to be similar in GLILD, as it has shown the reported efficacy of Sirolimus, an mTORC1 inhibitor, in the treatment of two GLILD patients[59].

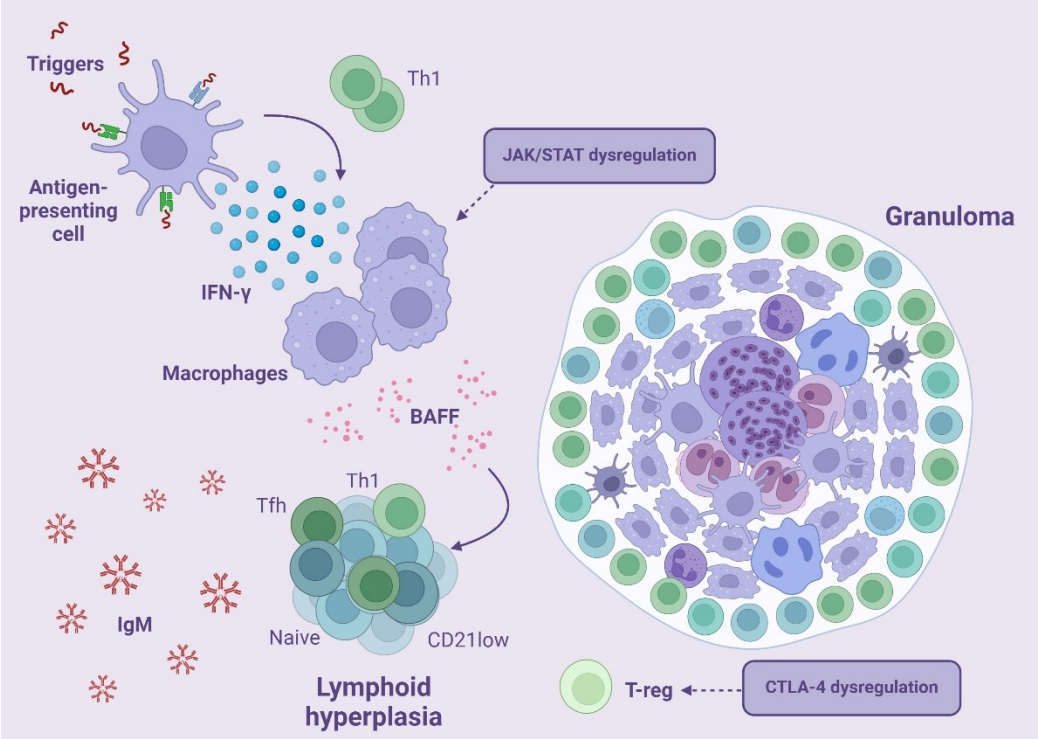


Figure 11: GLILD pathogenesis. The first trigger seems to be linked to chronic antigen exposure, eventually with predisposing environmental factors. Then, antigens are captured by antigen-presenting cells (APCs), which communicate with macrophages and T helper cells through interferon gamma (IFN-γ). Macrophages and other cell populations can produce B cell activating factor (BAFF), which encourages the lymphoid hyperplasia formation and IgM production; these cells can also be subjected to dysregulation, mainly regarding the JAK/STAT pathway. CTLA-4 is another factor that can facilitate lymphoproliferation and loss of tolerance. The final step is represented by the sarcoid-like non-caseating granuloma, which is a typical finding in GLILD patients. Created with BioRender.com.

2.5.4 Clinical manifestations

As it has already been highlighted, GLILD is a cryptic disease. Symptoms are usually vague (*e.g.* cough, shortness of breath) and the onset is insidious. A significant percentage of patients may be asymptomatic. Moreover, clinical manifestations often appear when the condition is in a late stage, and the lungs are already compromised. The prognosis is worse than in CVID non-GLILD patients, as aforementioned[49].

Some symptoms may be correlated to GLILD's collateral conditions: cytopenias are usually present in GLILD patients, in particular immune thrombocytopenia (ITP); splenomegaly is also quite a common finding.

2.5.5 Classifications

GLILD does not have a defined classification, as it is an umbrella definition under which different ILDs are collected. Nevertheless, given the fact that GLILD is a complication of CVID, it can share the same classifications as CVID.

So, it is possible to categorize GLILD patients using either the Chapel phenotypic classification or the EUROclass classification, both of which were described in the *paragraph 2.3.2*.

2.5.6 Biomarkers and possible predictors

Many studies have been analysing GLILD biomarkers as predictors of disease onset or progression (*see Figure 12*).

Clinical manifestations can be of use, but they usually appear in later stages. Splenomegaly and widespread lymphadenopathy are the main findings, but also polyarthritis may have a role. Other symptoms may be expressions of an underlying cytopenia, whose main percentage is represented by Immune Thrombocytopenia (ITP).

On the other hand, laboratory biomarkers may also be important predictors: elevated IgM, decreased IgG or IgA, cytopenias, low B or T cell markers are the main factors.

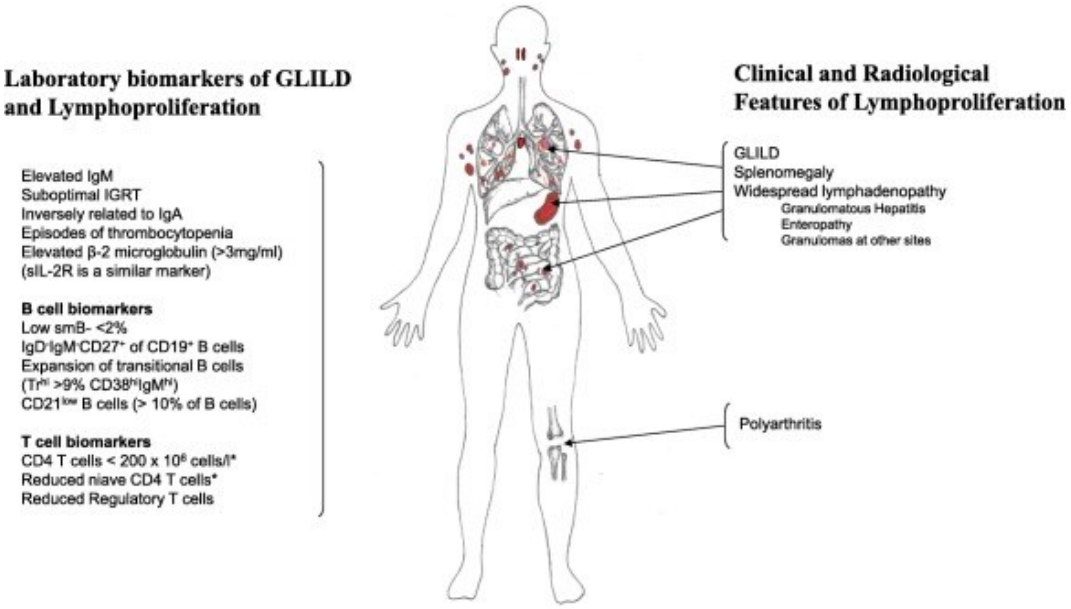


Figure 12: Clinical and laboratory risk factors for granulomatous lymphocytic interstitial lung disease (GLILD)[60]

As aforementioned, HLADR+CD4+ T cells, CD57+CD8+ T cells, $CD21^{low}$ B cells and BAFF are deeply involved in the pathogenesis of CVID and GLILD[51].

Additionally, serum markers of T cell activation and exhaustion (*e.g.* sCD25, sTIM-3, IFN-γ, TNF) may have a role in defining the development of the disease[52].

Given these biomarkers and clinical findings, many studies have tried to define which parameters have a diagnostic or prognostic significance.

Unfortunately, the question is still open, as there has not been an agreement yet.

A 2016 study conducted on thirty-four GLILD patients concluded that hypersplenism and polyarthritis seem to be strong risk factors for GLILD[61].

However, a more recent study, based on twenty-six cases, claimed that splenomegaly, history of ITP or AIHA and percentage expansion of $CD21^{low}$ B cells may be useful to identify high risk groups[62]. Moreover, a diagnostic algorithm was proposed (*see Figure 13*).

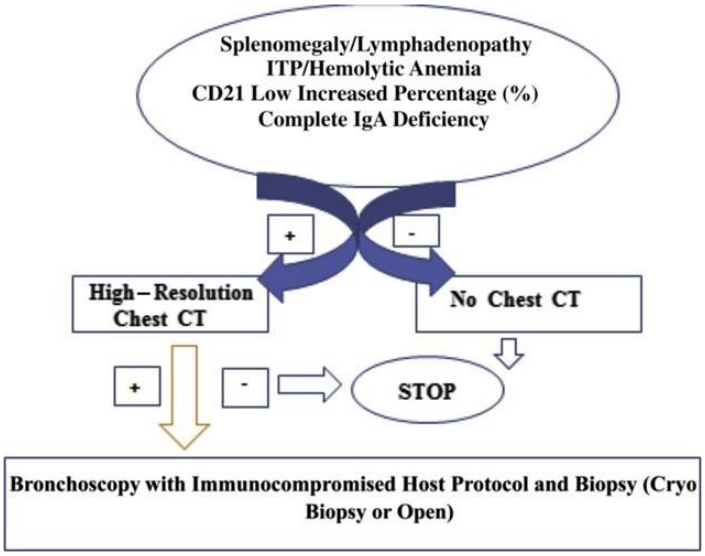


Figure 13: Proposed algorithm for the evaluation of CVID patients at risk for GLILD[62].

A few years later, an Italian study suggested that a diagnostic tool for early identification of GLILD should have included a combination of two clinical parameters (splenomegaly and autoimmune cytopenia), one lung function index (DLCO %) and one immunologic variable (CD21^{low} B cells%)[63] (*see Figure 14*).

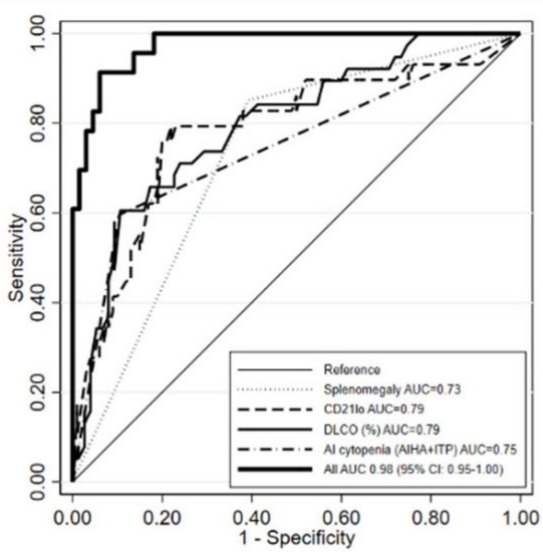


Figure 14: ROC curve and area under the curve (AUC) of a proposed GLILD diagnostic score[63].

Recently, another study was conducted, analysing fifty patients in a referral unit for primary immunodeficiencies. The results highlighted that lymphadenopathies, splenomegaly, Baumann's GLILD score (which evaluates the number of pulmonary lobes with specific alterations) and $CD8^+$ cell count could be the best possible predictors.[64]

Another scoring system, the Hartmann score, has been suggested. It has some small differences from the aforementioned Baumann score, and it is mainly based on a cystic fibrosis-CT scoring method. The reproducibility of this score has appeared to be slightly better than that of the Baumann method[65].

In conclusion, currently there still is a lack of evidence-based guidelines and wider studies are needed.

2.5.7 Diagnosis

GLILD is still a partially unknown condition, thus consensus diagnostic criteria are still required. At the moment, for example, histologic evidence of GLILD is needed for diagnosis[47], but new studies are reconsidering biopsies' crucial role in the diagnostic process. Moreover, it is not always clear whether to include a specific ILD occurring in a CVID patient under the umbrella diagnosis of GLILD[66].

At the present time, diagnosis revolves around three main approaches[67]:

- Imaging (*e.g.* HRCT, PET-CT)
- Pulmonary function tests (PFTs)
- Histology (*e.g.* lung biopsy)

Analysis of broncho-alveolar lavage (BAL) can also be performed, primarily to exclude infections.

Radiology is the most used method of investigation, as it first raises suspicion of GLILD. PFTs are also widely used, showing mainly a restrictive pattern and reduced gas transfer. Histology is commonly represented by lung biopsies (open or VATS) and trans-bronchial biopsies (TBBs).

Differential diagnosis for GLILD is represented by infections, lymphoma, sarcoidosis, cryptogenic organizing pneumonia (COP), lymphoid interstitial pneumonia (LIP) and other interstitial lung diseases [47].

2.5.7.1 Imaging

HRCT is an essential tool, as morphologic alterations generally occur earlier than functional dysregulation.

The main HRCT findings of CVID include diffuse bronchiectasis, bronchial wall thickening, atelectasis and air trapping[68], but the most peculiar aspects of GLILD are the presence of solid and semisolid nodules, diffuse ground-glass, fibrosis areas, and hilar and/or mediastinal lymphadenopathy (*see Figure 15*) [69 23 70].

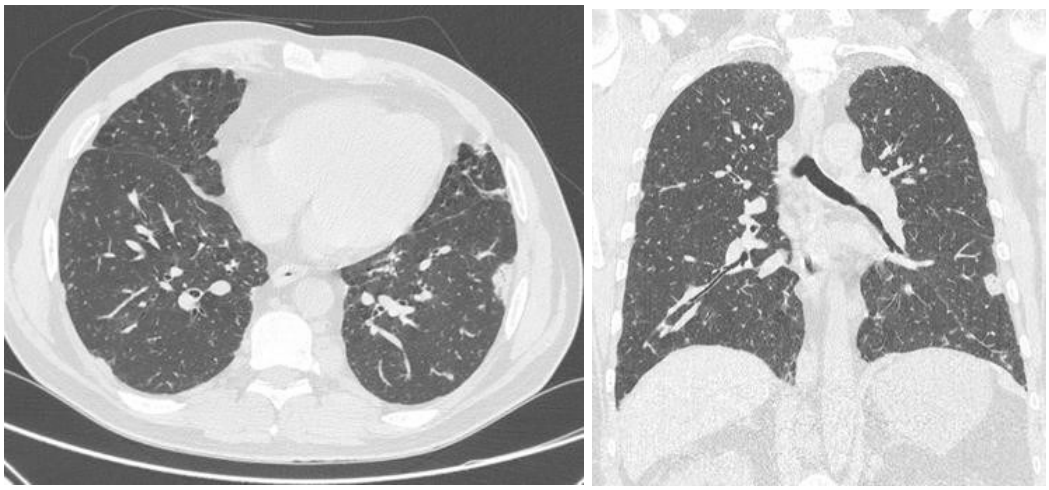


Figure 15: Typical HRCT pattern of one of our GLILD patients.

Nodules need to be scrupulously observed, in order to conduct an accurate differential diagnosis. The main conditions that can mimicry GLILD's nodules are lymphomas and sarcoidosis. Sarcoidosis, in particular, is frequently compared to GLILD, because the both of them present non-necrotising systemic granulomas and have preferential involvement of lungs and lymph nodes. Moreover, both sarcoidosis and GLILD are based on profound immune dysregulation, probably due to an impairment in antigen clearance[71]. However, there are some features that allow to discriminate: nodules in GLILD patients are usually larger (>3 cm) and have a predominant basal distribution, while

sarcoidosis is characterized by micronodules, frequently confined to the upper lobe (*see Figure 16*).

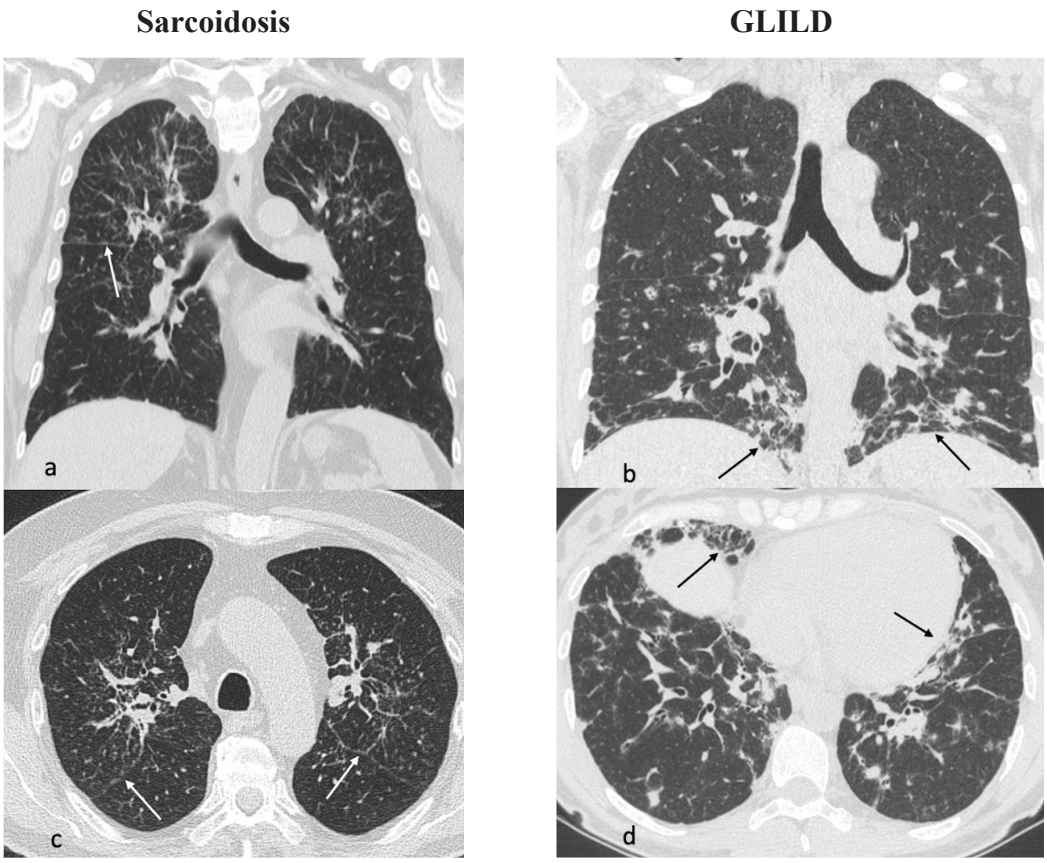


*Figure 16: HRCT of our two patients, one with sarcoidosis (**a,c**) and one with GLILD (**b,d**). It appears clearly that sarcoidosis has a prevalent medium-upper involvement, with multiple perylimphatic nodules (*white arrows*) and perihilar fibrosis. On the other hand, GLILD has a lower distribution, with consolidations and nodules; reticulations (*black arrows*) with some signs of fibrosis can also be seen*[71].

Recent evidence supports the possible role of lung magnetic resonance imaging (MRI) in diagnosing and monitoring lung disease in GLILD patients[66]. This would be an important improvement, reducing the patients' exposure to ionizing radiation.

Another important tool for the monitoring of GLILD is fluorodeoxyglucose (FDG)-positron emission tomography-computed tomography (PET-CT). This technique is generally used to observe the development of the condition on a systemic scale and to assess the response to treatment. It can detect both morphological and functional changes, but its strength is in the possibility to compare the metabolic activity of the same area over time, to eventually identify

new inflammatory areas. Moreover, it can give a clearer picture of the extrapulmonary dissemination of the pathology, addressing an eventual therapy (*see Figure 17*).

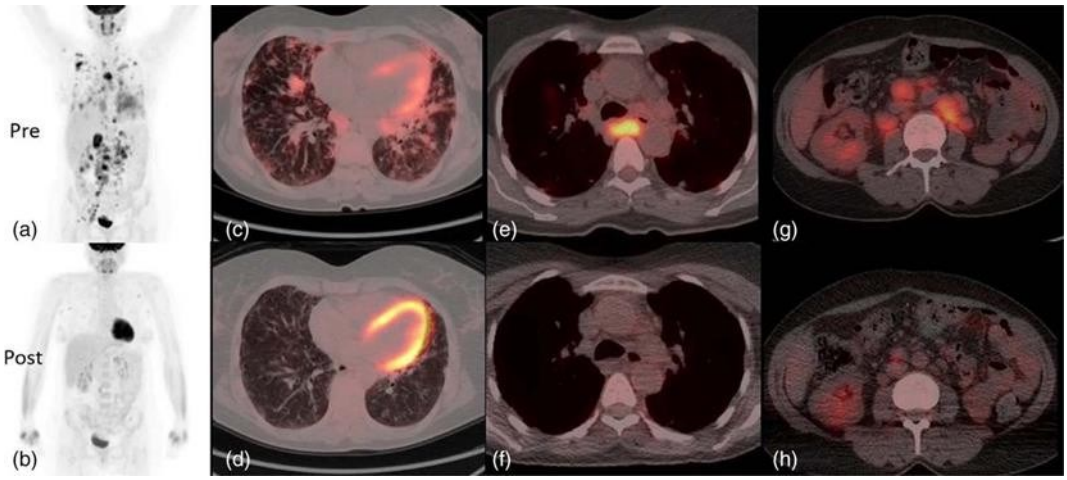


Figure 17: 2-[(18)F]-fluoro-2-deoxy-d-glucose positron emission tomography and computed tomography (FDG PET-CT) pre- and 3 months post- specific GLILD immunosuppressive treatment with rituximab and mycophenolate[60].

In conclusion, even if histology is always required, imaging can be reliable in determining the severity of the condition: it has been observed that patients with progressive GLILD, defined by deteriorating pulmonary function, have significantly greater pathology on pulmonary CT and FDG-PET-CT scans as compared to patients with stable disease[72]. This means that there is actually a correlation between the morphologic and functional aspects of the disorder.

2.5.7.2 Pulmonary Function Test

Pulmonary function tests (PFTs) are assessment tools widely used to analyse the impact of GLILD on lung functionality. Usually they include spirometry, measurement of diffusing capacity of the lung for carbon monoxide (DLCO) and assessment of static lung volumes (*e.g* total lung capacity, TLC, and residual volume, RV).

However, the results can be very different from one patient to another and vary from normal to severely impaired: GLILD patients' PFTs can present a restrictive pattern, an obstructive pattern or a mixed result. On the other hand, gas transfer is usually low in the majority of the cases[67].

2.5.7.3 Histology

Biopsies represent an excellent tool in the diagnosis of GLILD, as they give information on lymphocytic infiltration and/or granulomas formation. Moreover, histology is the last part of the multi-step process that leads to the acknowledgement of the disease, so it is essential that the test is conducted correctly.

The gold standards are the open lung biopsy and the video-assisted thoracoscopic surgery (VATS) biopsy[66], but they are invasive and they present several side effects. In particular, it is possible that patients that undergo these procedures develop infections, prolonged air leakage, persistent pain or even death (under 2% of cases)[73]. These risks are higher in patients with severely compromised lung function[74].

Transbronchial biopsy, on the other side, may be used to exclude different diagnosis, such as infections and lymphomas. The main limit of this procedure is that it is not sensitive enough to give acceptable results for a GLILD diagnosis, due to the small sample size.

A biopsy of another affected site can be considered, as it may be less invasive and less hazardous for the patient. Lymph nodes, for example, are a safer alternative.

GLILD has a very heterogeneous histologic presentation (*see Figure 18*). Peribronchiolar and interstitial lymphocytic infiltration, sarcoid-like non caseating granulomatous hyperplasia, and organizing pneumonia are consistent features. Lymphoid hyperplasia is deeply connected to the granulomas, either because the granuloma is surrounded by lymphocytic infiltrate or because the granuloma itself spreads out around the reactive follicle. Extensive organizing pneumonia and interstitial fibrosis with architectural remodelling have also been seen in a significant proportion of patients[75].

Lymph nodes in GLILD usually present ill-defined germinal centres, eventually with an altered polarisation and infiltrated $CD8^+$ T-cells. Class switched plasma cells (PCs) are usually absent or severely reduced, and if present, rarely found in

the medulla of the lymph node[76]. This may be an indicator of germinal centre failure.

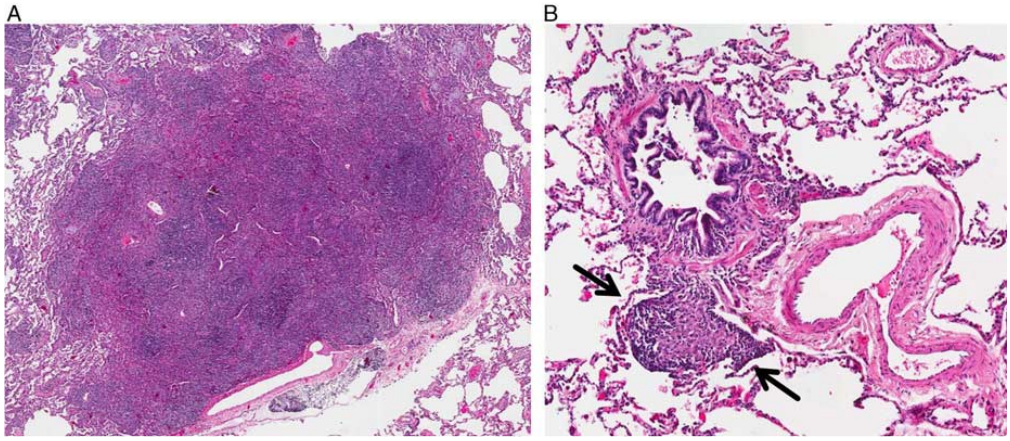


*Figure 18: A, Hematoxylin and eosin stain of GLILD shows nodular areas of lymphoid hyperplasia. Underlying architecture is preserved despite density of the lymphocytic infiltrate. B, In the setting of granulomatous infiltration, a non-necrotizing granuloma (*arrows*) is immediately adjacent to the bronchovascular bundle[42].*

The goal set for the future is to remove biopsies from the diagnostic set up, and the reason behind this can be found in the aforementioned side effects. Nevertheless, at the moment it is impossible to have a clear diagnosis of GLILD without a biopsy.

2.5.7.4 Bronchoalveolar lavage (BAL)

The analysis of bronchoalveolar lavage fluid (BALF) is recommended for microscopy, bacterial, mycobacterial and fungal culture[47].

On the contrary, flow-cytometric analysis is not reliable, as it has not been proved a significant role in the diagnostic process. Anyway, it is usually performed to gain more information.

The main features of GLILD that have been studied at BALF analysis so far are the expansion of T cells and B cells and, in particular, of $CD21^{low}$ B cells, subpopulation that has been recently studied for its potential diagnostic[77].

2.5.8 Management

There is not a systematic protocol for GLILD management and treatment, as official guidelines lack. Many studies have been conducted, but there has not been a consensus on the most effective strategy to use. Different articles describe small studies of monotherapy with glucocorticoids or other immunosuppressants, rituximab monotherapy or rituximab plus azathioprine, abatacept, or hematopoietic stem cell transplantation (HSCT). Treatment response rates vary widely, and mortality, especially among immunocompromised patients, is high.

It is clear that, as the disease has a systemic involvement, a multidisciplinary approach is needed. Moreover, as different patients may develop quite different organ distribution, therapy should be as personalized as it can.

Firstly, before starting a specific therapy, IgG replacement therapy is suggested, to optimize the immunoglobulin levels of the patients. The injection can be intravenous (IVIG) or subcutaneous (SCIG). Subcutaneous route presents some differences from the subcutaneous: more frequent and smaller doses, lower peaks and higher troughs, more patient autonomy, decreased systemic adverse effects[78], and the lack of a requirement for vascular access[79]. Both the procedures seem to enhance survival and to reduce severe and invasive infections[80]. However, patients might still develop chronic infection-related lung disease (CLD)[66]. Additionally, the long-term effect of intravenous or subcutaneous Ig on the disease progression is still unclear[47].

Adjunct therapies, as Azithromycin prophylaxis and pulmonary rehabilitation, in some cases have a great role in controlling GLILD complications. On top of that, new IgA and IgM enriched Ig preparations will soon be available[66].

It is not clear which may be the appropriate timing for treatment initiation, but the choice should be based on the presence and severity of symptoms and on the grade of lung impairment.

The first line of treatment, after the Ig replacement therapy, is represented by oral steroids[47 81]. They have a great impact in improving gas transfer in GLILD, nevertheless patients often relapse. Mycophenolate may be used as a maintenance

therapy, as it seems to be associated with long-term effectiveness and permits weaning of corticosteroids[82].

The second line of treatment is generally agreed to include azathioprine, mycophenolate and rituximab[47].

Azathioprine and mycophenolate are part of the family of disease-modifying antirheumatic drugs (DMARDs), which may be an option to reduce the usage of corticosteroids. Other exponents are cyclosporin, hydroxychloroquine and methotrexate. Azathioprine's mechanism of action, in particular, works directly against T cells, which appear to have an undiscovered role in the pathogenesis.

Rituximab, on the other hand, affects exclusively the B cells, interacting with their CD20. Symptom burden and quality of life have significantly improved in most of the treated patients, lung function has increased, and lung CT scan findings have decreased [83]. Therefore, rituximab can be considered a widely effective option[84]; in fact, it has been observed that relapses are less frequent in patients treated with rituximab-containing treatment regimens if compared to corticosteroid monotherapy [85]. However, there are currently no data on the long-term efficacy and side effects of rituximab and other second-line therapeutic options[86].

A combination therapy of rituximab and azathioprine has been proposed, to target B and T cells at the same time (*see Figure 19*). This therapy has been studied to be effective, determining an improvement of both lung function and radiographic findings[87].

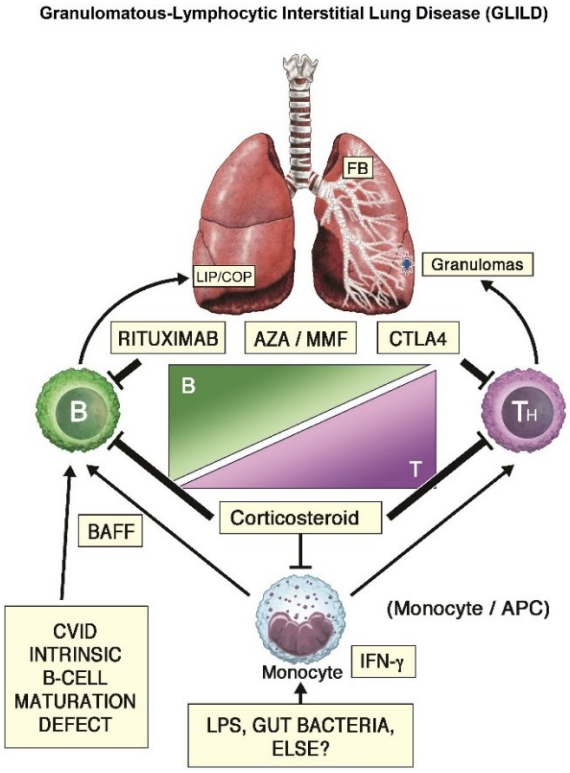


Figure 19: Cooperation between B and T cells inhibitors in the treatment of GLILD. GLILD in CVID seems to be driven by increased IFN-γ production, which stimulates macrophages, via STAT1 signalling, to secrete more BAFF. Together with CVID-intrinsic B-cell maturation defects, this seems to lead to pulmonary lymphoproliferation. Conversely, the role of local T cells in the exact etiopathogenesis of GLILD remains obscure, but a short course of glucocorticoid therapy, rituximab monotherapy, or rituximab plus azathioprine (AZA) or mycophenolate (MMF) treatment seems at least partly effective[88].

Other possible options of treatment may be represented by tacrolimus, sirolimus, abatacept and anti-TNF agents (*e.g.* Infliximab[89]). Also, Belimumab, an anti-BAFF, might be a promising alternative treatment approach, considering the aforementioned role of BAFF in the pathogenesis of the disease.

Hematopoietic stem cell transplantation (HSCT), in addition, is a promising alternative to pharmacological treatment of GLILD, because it has the potential to resolve the underlying pathology, not just to contain symptoms. Anyway, the risks for this procedure are high[85].

Finally, in the later stages of GLILD, when the lung function has critically decreased and the symptoms are severe, the only remaining option is lung transplantation[90 91].

3. Aims

GLILD is a relatively unknown entity, no large cohort studies are available and little is known about its clinical behaviour and appropriate management. The aims of this retrospective study are:

- To describe clinical behaviour, survival and mortality in an Italian cohort of GLILD patients;
- To analyse pulmonary function trajectories in GLILD patients, trying to find parameters that have a significantly relevant correlation with GLILD development and prognosis;
- To give a better understanding of GLILD's clinical manifestations, trying to discern the different phenotypes in which the disease can present;
- To have a general overview on different therapeutic strategies and on how much they impact on patients' condition.

4. Materials and methods

4.1 Study population

We considered all patients with GLILD followed by the Referral Center for adult primary immune deficiency of Ca' Foncello Hospital in Treviso, Policlinico Umberto I, Sapienza University of Rome, Federico II University Hospital in Naples and Cagliari University Hospital.

The Inclusion criteria were:

- Histologic or clinical-radiologic diagnosis or suspicion of GLILD (given the absence of official diagnostic guidelines);
- At least 18 years of age;
- Availability in the clinical records of the reports of laboratory and radiological parameters regarding GLILD diagnosis and follow-up (IgG, IgA and IgM concentrations at diagnosis, eventual cancer or decease);
- Signature of the informed consent.

On the other hand, no specific exclusion criteria were defined.

GLILD patients were further divided into 2 groups. A subgroup of patients with negative prognosis was selected to be part of the "**case group"**. These patients presented at least one of these characteristics as inclusion criteria:

- Need for immunosuppressive therapy other than steroids (*e.g.* DMARDs, Rituximab);
- Occurrence of lymphoma;
- Death.

All the GLILD patients that did not meet the criteria were enrolled in the positive prognosis group (**control group**).

4.2 Data collection

This study was conducted via retrospective review of medical records of cases and controls from the referral centres of Treviso, Naples, Rome and Cagliari. The data collected for each patient, when available, were:

- Personal information: age, sex, year of first symptoms and year of diagnosis (both symptoms and diagnosis of either CVID or GLILD were analysed);
- The environmental exposition to chronic antigens (smoke, occupational exposition);
- The value of IgG, IgA and IgM at diagnosis and at last available control;
- The response to tetanus vaccine and pneumococcal vaccine;
- Presence of comorbidities: splenomegaly (with eventual splenectomy), bronchiectasis, autoimmunity (with particular attention to ITP, AIHA and other cytopenias), cancer (either hematologic or solid);
- The type (IvIg or ScIg) and the dose of immunoglobulin replacement therapy at diagnosis and at last available control;
- Eventual histologic diagnosis of GLILD, in particular distinguishing lung biopsy from lymph node biopsy or other organs;
- Presence of extrapulmonary involvement (*e.g.* hepatopathy, lymphadenopathy);
- The analysis of B cell subtype according to Euroclass flow-cytometric classification (considering the percentage and the absolute number of CD19, CD27+IgM-IgD- switched memory B cells, CD27-IgM+IgD+ naïve B cells, CD27+IgM+IgD+ marginal zone B cells, $CD21^{low}$ $CD38^{low}$ activated B cells, CD38++IgM++ transitional B cells and CD38+++IgM- plasmablasts);
- The analysis of differential cell count and lymphocyte subtypes on BALF (considering the percentage of macrophages, neutrophils, eosinophiles,

lymphocytes; of this last population, CD3+, CD19+, the CD4+/CD8+ ratio were analysed, with eventually additional B cell subtypes);

- Genetic tests' results;
- HRCT evaluation, considering these parameters: ground glass opacities, nodules, bronchiectasis, lymphadenopathy;
- Organ involvement based on ^{18}FDG-PET-CT (in particular supradiaphragmatic and subdiaphragmatic lymph nodes, lungs, spleen and liver);
- Every pulmonary function test ever performed by the patient, in particular the following functional lung parameters: FEV1, FVC, TLC and DLCO;
- Immunosuppressive therapy (*e.g.* DMARDs, steroids, rituximab);
- Eventual death (and its cause);

The study was conducted according to the Helsinki declaration and current Italian regulation and approved by the local Ethical Authority (Study 1342 CE Marca).

4.3 Statistical analysis

Data were collected in a Microsoft Excel database and then analysed with the programmes “Jamovi 2.3.28” and “GraphPad 10”.

Descriptive statistic was used to investigate the demographic characteristics of the population, the variety of clinical manifestations, the humoral and radiological findings, the treatment options, and the deaths that had occurred.

Given the small sample size, non-parametric analysis was used. The quantitative variables were summarized with the median and interquartile range, while the qualitative variables with the frequency percentages.

Comparisons of continuous parameters were accomplished with the use of Mann-Whitney U-test, instead the comparisons of categorical parameters were made with the Fisher’s exact test. For the analysis of variation over time of some variables within the same group we used the Wilcoxon matched-pair signed rank test.

For mortality rate, we performed a survival analysis, to obtain a Kaplan-Meier curve. For pulmonary function, the normal distribution with z-scores and percentiles was displayed, representing the comparison between the sample we analysed and the general population.

We performed a logistic regression analysis to detect a possible relation between our variables and GLILD prognosis. ROC curves were used to analyse the prognostic potential of single parameters.

5. Results

5.1 Description of study population

The population we analysed included 64 patients from multiple centres.

The sample included:

- 28 patients from Ca' Foncello Hospital in Treviso (one of which was referred by Ancona University Hospital);
- 23 patients from Policlinico Umberto I, Sapienza University of Rome;
- 9 patients from Federico II University Hospital in Naples;
- 4 patients from Cagliari University Hospital.

All of these patients fulfilled the above-mentioned inclusion criteria.

Among them, we selected 38 patients (59% of the sample) to be enrolled in the "negative prognosis group" (**case group)**.

All the patients that did not fulfil the criteria (26 people, representing 41% of the sample) were enlisted in the **control group**. These patients appeared to have a milder phenotype, so they were considered as the "positive prognosis group".

5.1.1 Demographic characteristics

The demographic characteristics of the whole cohort are summarized in the following *Table II.*

Age	50.5 (38.75-60.25)
Age at first symptoms of CVID	30 (16.75-37.25)
Age at first symptoms of GLILD	39 (29-48)
Age at diagnosis of CVID	38 (26.25-46.75)
Age at diagnosis of GLILD	44 (34-56)
Diagnostic delay of CVID	5 (2.75-13)
Diagnostic delay of GLILD	2 (0-7.75)

Table II: Demographic characteristics of the whole cohort

Median and interquartile range of age, age at diagnosis, age at first symptoms and diagnostic delay of respectively CVID and GLILD in the sample.

The general population did not present surprising characteristics compared to the Italian CVID population, except for the sex distribution, which was relatively unbalanced towards female sex (*see Figure 20*). We compared the data with the sex distribution from the whole CVID cohort of the participating centers, that appeared to have a more balanced sex distribution (F=56.7%, M=43.4%).

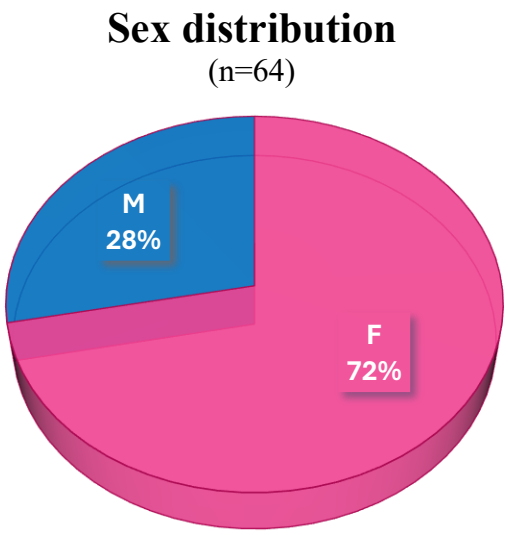


Figure 20: Sex distribution of the whole GLILD sample.

Focusing on antigenic exposure, 14 patients were former smokers, while one was an active smoker. This group had a median of 235 cigarette packs/year. Moreover, 6 patients were considered to have had professional exposure in their life, mainly due to jobs in the mechanic field.

5.1.2 Biopsies and histological findings

Of all our patients, 29 underwent at least one biopsy, whose sites are summarized in the following *Figure 21*. In total we obtained 43 biopsies, given the fact that some patients had more than one biopsy.

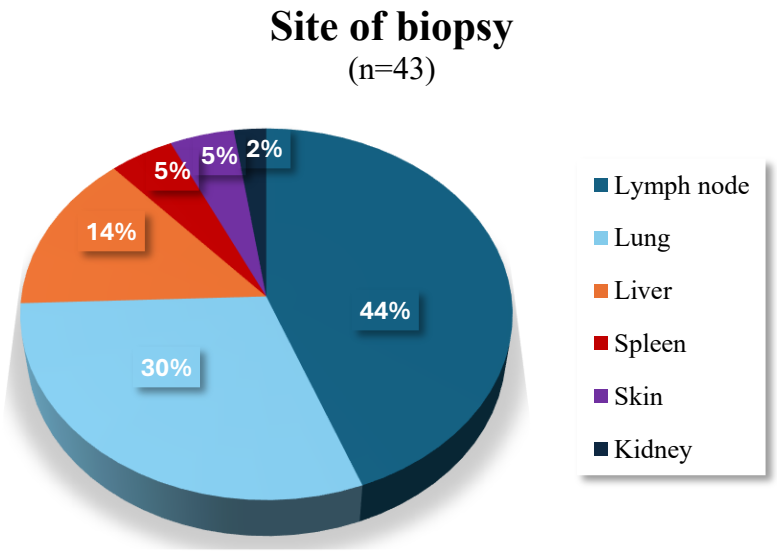


Figure 21: Distribution of sites biopsied for each patient. When a patient performed more than one biopsy in the same anatomic system (for examples two biopsies in two lymph nodes), it was considered as one.

For each biopsy, we searched for three main characteristics:

- Fibrosis;
- Follicular lymphoid hyperplasia;
- Granulomas.

Lung biopsies and lymph node biopsies were analysed separately, as higher numbers of exams were performed. The other biopsies were all collected together, as they had smaller numbers. The results are summarized in the following *Figure 22, 23 and 24*.

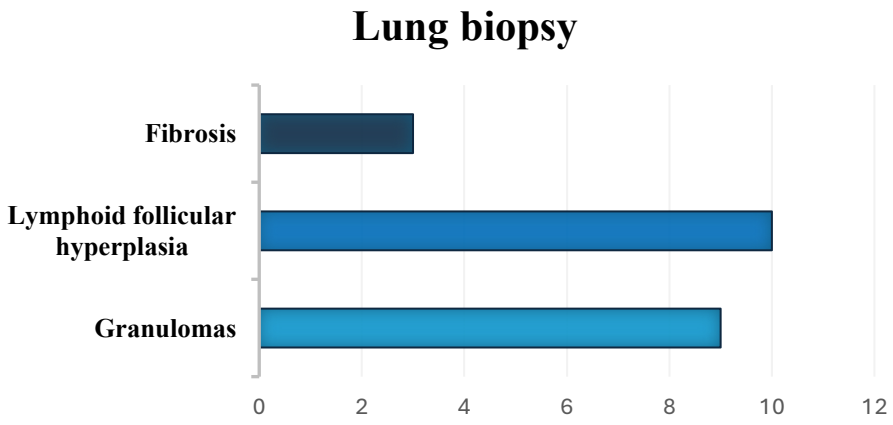


Figure 22: Main findings from lung biopsies.

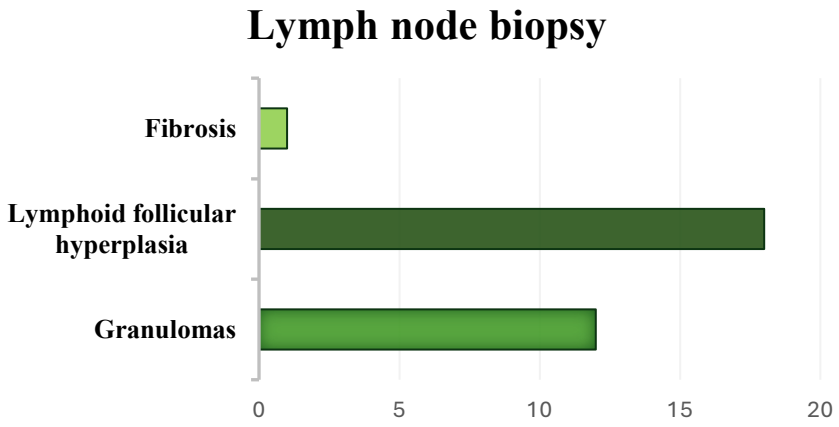


Figure 23: Main findings from lymph node biopsies.

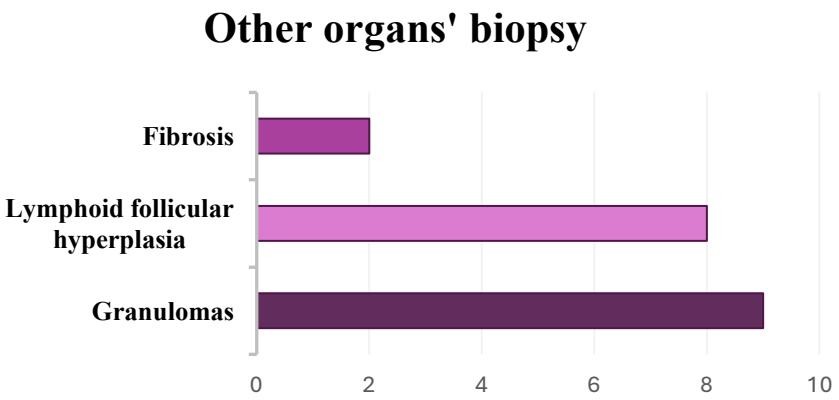


Figure 24: Main findings from other organs' biopsies.

5.1.3 Laboratory parameters

5.1.3.1 Immunoglobulin serum levels and Ig replacement therapy

Firstly, the immunoglobulin serum levels (IgG, IgA, IgM) at diagnosis of CVID were collected. Data were available for 57 patients, showing a median of 236.0 mg/dL for IgG (128.0-375.0), 10.0 mg/dL for IgA (5.5-24.0) and 18.0 mg/dL for IgM (9.0-44.3).

Then, we focused on the administration of immunoglobulin replacement therapy (IgRT). All 64 patients were under IgRT, and 50 of them got antibiotic prophylaxis (78%). The main route for administration of IgRT was subcutaneous (51 patients, 80%).

The data we searched for were:

- First and last available dose of IgRT;
- First and last available Ig serum level under Ig replacement therapy (IgRT), also defined as Ig trough level (IgTL).

The median of IgRT dose was 376.0 mg/kg/4 weeks (325.0-430.0) at first and 401.0 mg/kg/4 weeks (353.0-474.0) at last available measurement, with a significant increase ($p=0.002$**). IgTL median was 597.0 mg/dL (412.0-743.0) at first available measurement and 738.0 mg/dL (628.0-877.0) at last, again with a significant variation ($p<0.001$***).

5.1.3.2 Analysis of serum B and T cell subpopulations

We analysed B and T cell subpopulations from the serum of 56 patients, on the basis of data availability.

In particular, T cells percentage (CD3+%) was calculated, along with the percentages of T helper cells (CD4+%), T cytotoxic cells (CD8+%) and their ratio (CD4+/CD8+).

Then, we considered the B cells percentage (CD19+%) and we collected data regarding the B-cell immunophenotyping according to the EUROclass protocol.

The population's results are presented in *Table III*:

CD3+%	79.00 (72.00-84.00)
CD4+%	46.00 (39.00-51.30)
CD8+%	28.50 (21.90-37.50)
Ratio CD4+/CD8+	1.27 (0.85-1.82)
CD19+%	8.00 (5.85-12.00)
Naïve %	81.2 (70.0-88.3)
Marginal zone %	4.50 (2.00-14.80)
Switched memory %	1.80 (0.85-2.67)
Transitional %	1.90 (0.95-4.05)
Plasmablasts %	0.90 (0.20-1.47)
CD21^{low} %	13.20 (4.65-30.30)
CD3+CD57+ %	14.00 (6.10-21.50)

Table III: B and T cells subpopulations

Median and interquartiles of B and T cell subpopulations. Naïve B cells = CD27-IgM+IgD+; marginal zone B cells = CD27+IgM+IgD+; switched memory B cells = CD27+IgM-IgD-; transitional B cells = CD38++IgM++; plasmablasts = CD38+++IgM-; activated B cells = CD21lowCD38low.

5.1.3.3 Analysis of the bronchoalveolar lavage fluid (BALF)

Of our sample, 36 patients performed an analysis of the differential cell count and lymphocyte phenotyping on bronchoalveolar lavage fluid (BALF).

The median cell count was 155.00/mm^3 (81.00-279.00). The other results are expressed in the following *Table IV*.

Macrophages %	41.00 (11.50-66.30)
Neutrophils %	12.00 (2.75-39.50)
Eosinophils %	0.00 (0.00-0.50)
Lymphocytes %	25.00 (13.50-50.00)
CD3+%	89.70 (85.80-95.00)
CD4+/CD8+ ratio	1.45 (1.02-2.58)
CD19+%	2.92 (1.15-7.84)
Switched memory %	11.80 (2.83-13.30)
Naïve %	13.30 (2.83-22.50)
Marginal zone %	1.85 (0.40-3.15)
CD21^{low} %	75.50 (61.50-80.80)
Transitional %	0.00 (0.00-0.00)

Table IV: BALF features

Median and interquartiles of BALF features.

5.1.4 Pulmonary function tests

We collected every pulmonary function test (PFT) that each patient performed, comparing then the first and the last available PFT.

The parameters we considered were:

- Forced expiratory volume in 1 second (FEV1);
- Forced vital capacity (FVC);
- Total lung capacity (TLC);
- Diffusing capacity of the lung for carbon monoxide (DLCO).

The analysis of the populations gave the results listed in the *Table V* below.

First PFT	
FEV1 (L)	2.65 (2.16-3.29)
FEV1%	86.00 (75.00-99.00)
FVC (L)	3.37 (2.64-4.11)
FVC%	87.00 (74.00-99.00)
TLC (L)	4.91 (2.96-5.44)
TLC%	85.00 (79.00-96.30)
DLCO (mL/min/mmHg)	18.5 (14.7-22.9)
DLCO%	77.00 (66.00-91.00)

Last PFT	
FEV1 (L)	2.58 (2.08-3.05)
FEV1%	86.0 (71.80-93.80)
FVC (L)	3.26 (2.80-3.87)
FVC%	87.50 (74.30-102.00)
TLC (L)	4.79 (4.19-5.34)
TLC%	85.00 (74.80-100.00)
DLCO (ml/min/mmHg)	17.1 (11.8-20.1)
DLCO%	75.00 (59.00-89.00)

Table V: Lung function parameters of the population

Median and interquartiles of FEV1, FVC, TLC and DLCO and percentage of predicted of the study population at first and last available PFT

No statistically significant variation was found in the evaluated parameters between first and last PFT. However, for treated patients, last PFT were performed after treatment, thus possibly masquerading the pre-treatment decline. We focused then on DLCO, observing that its values were interestingly lower than general population. The difference can be seen in the following *Figure 25*.

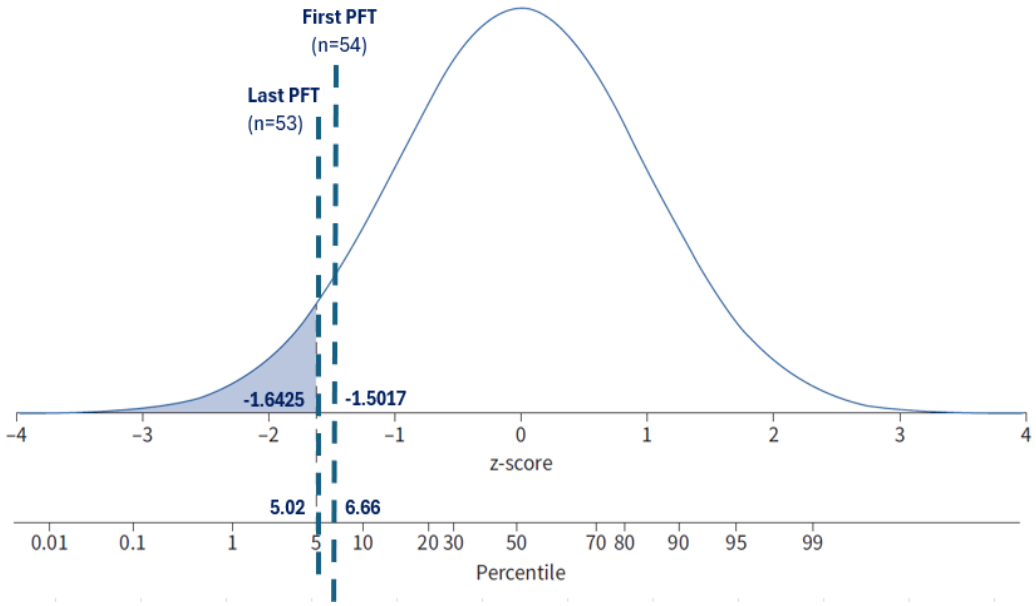


Figure 25: DLCO distribution at first and last available PFT, in comparison to the normal DLCO distribution. The graph was modified from the article "ERS/ATS technical standard on interpretive strategies for routine lung function tests"[92].

5.1.5 Genetics

Genetics testing results were available for 22 patients: out of them, 10 were negative. Focusing on the 12 positive tests, we obtained 13 different mutations, as some patients had more than one. The most common finding was the mutation of TACI, the BAFF receptor already mentioned in *Paragraph 2.5.3*. TACI is part of the TNF receptors superfamily, and interestingly we noticed that also other two TNF receptors were obtained from the genetic tests: TNFRSF12 and TNFRSF1A. Another common feature was the mutation of CTLA4, also already mentioned in *Paragraph 2.5.3*. The results are summarized in *Figure 26*.

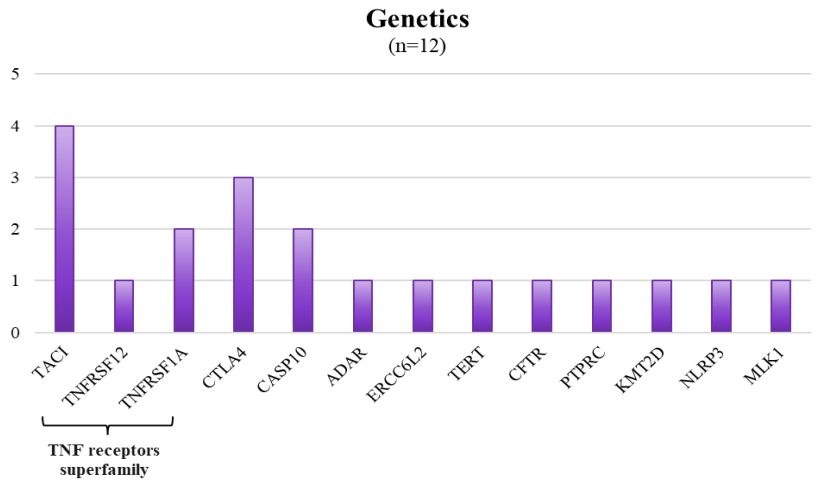
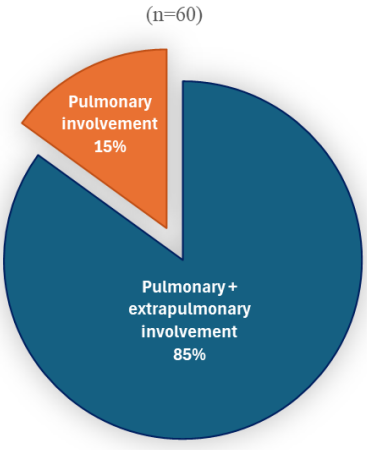


Figure 26: Genetic tests results

5.1.6 Clinical manifestations

From what is known so far, GLILD appears to be a lung manifestation of a systemic disease, rather than a lung disease. This characteristic was visible in our sample: as showed in *Figure 27*, the 85% of our population presented extrapulmonary involvement.

Figure 27: GLILD's dissemination



In particular, spleen involvement was very common (*see Figure 28*): 77% of our patients presented splenomegaly (defined as diameter >13 cm). Of this subgroup, 8 subjects (13% of the total) underwent splenectomy.

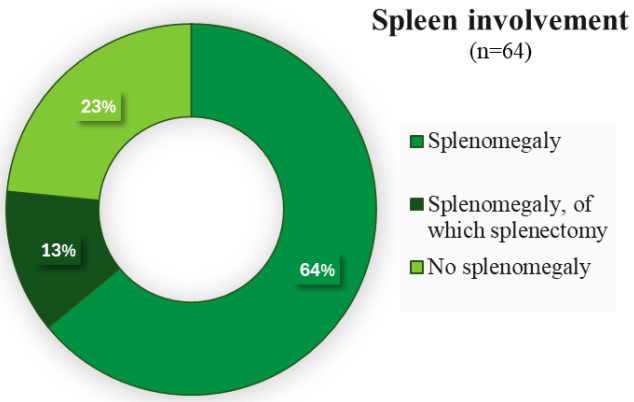


Figure 28: Spleen involvement in our population.

Then, we classified the patients according to the Chapel classification, mentioned in *Paragraph 2.5.5.1.* As expected, nobody was included in the first category ("infection only). As it is highlighted in *Figure 29*, the more common category was the one regarding "polyclonal lymphoproliferation" (III), since GLILD itself generally includes lymphoproliferation between its features, followed by "cytopenias" (II).

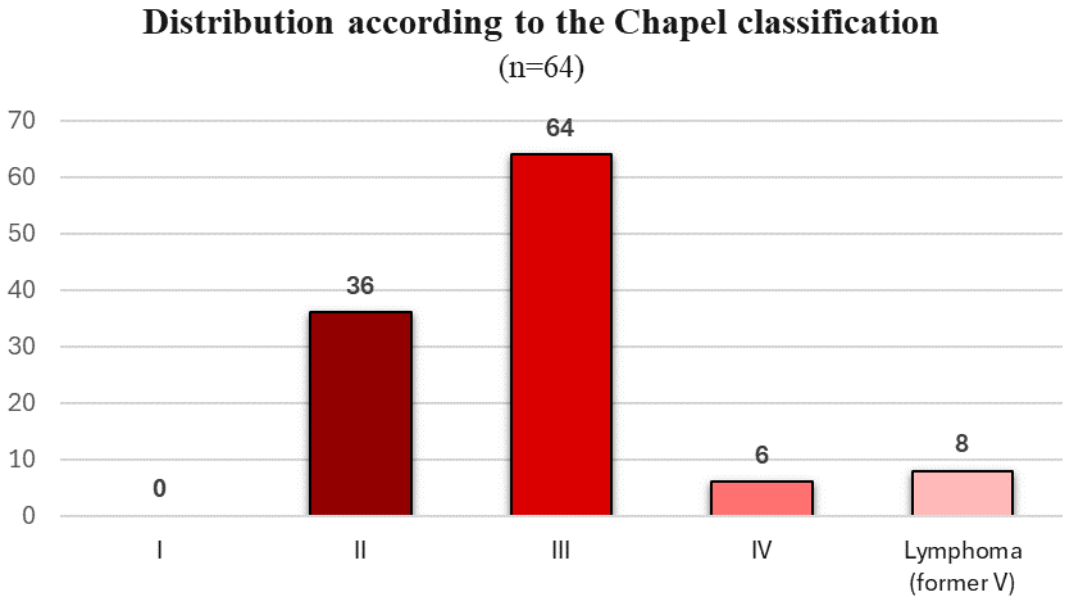


Figure 29: Chapel classification of the population.

Focusing on autoimmunity, we listed all the manifestations, and we collected them under groups, according to the organ involvement or the systemic dissemination.

For what it concerns organ autoimmunity (*Figure 30*), we also specified if some diseases were more common than others, but we generally preferred to enlighten the organ involved, rather than the singular disease. Hepatopathy was predominant in the cohort (representing 31% of the total).

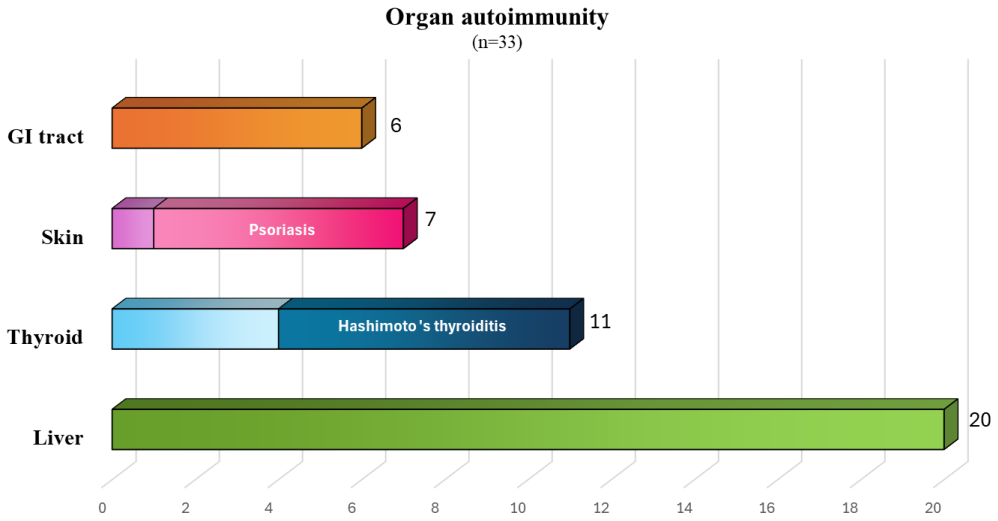


Figure 30: Organ autoimmunity in GLILD, divided by type of organ. Most common diseases are emphasized.

Afterwards, we considered systemic involvement. It appeared to be quite common, with the leading disease being immune thrombocytopenia (ITP), followed by autoimmune hemolytic anemia (AIHA), as summarized in *Figure 31*.

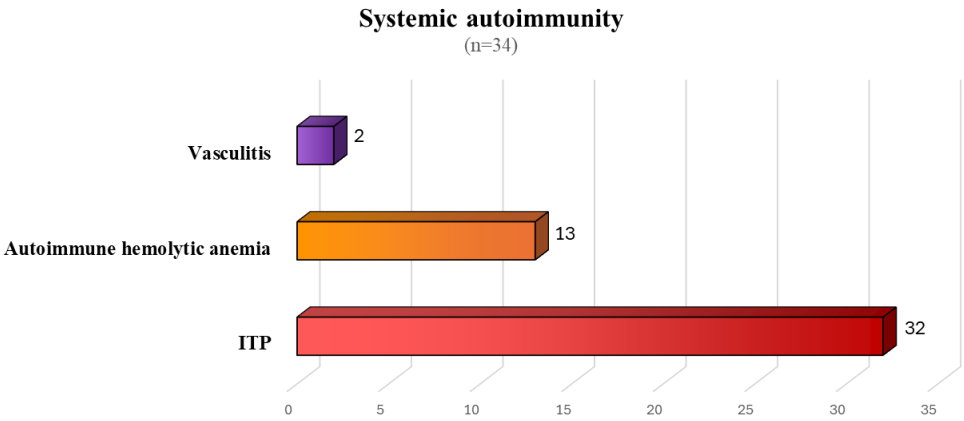


Figure 31: Main manifestations of systemic autoimmunity in GLILD.

Finally, we observed the complications, the main one of which was represented by malignancy. Out of 64 patients, 16 had history of cancer, half of which had solid cancer and half hematologic (*see Figure 32*), most of which were lymphomas.

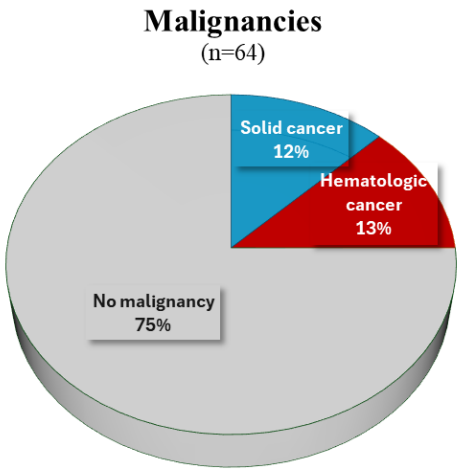


Figure 32: Prevalence of malignancies in the whole population.

5.1.7 CT and ^{18}FDG-PET-CT

We analysed the main findings of CT and ^{18}FDG-PET-CT of the whole population through the years. CTs from 52 patients and ^{18}FDG-PET-CTs from 33 patients were collected.

For CTs, we gained information about four characteristics:

- Lymphadenopathies;
- Bronchiectasis;
- Nodules;
- Ground glass opacities (GGOs).

For ^{18}FDG-PET-CTs, we investigated if there was an involvement of:

- Liver;
- Spleen;
- Lung;
- Subdiaphragmatic lymph nodes;
- Supradiaphragmatic lymph nodes.

The results are showed in the *Figures 33 and 34* below.

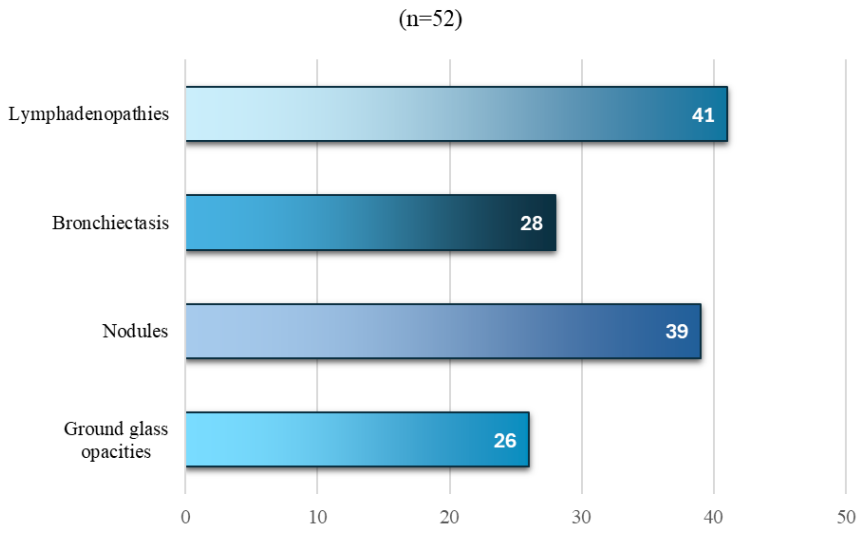


Figure 33: Findings from first available chest CT scan

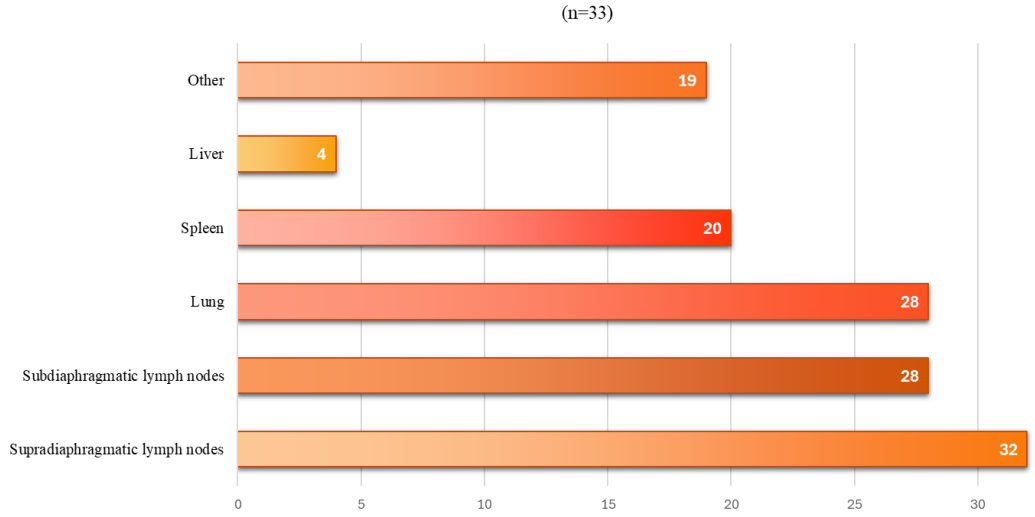


Figure 34: Organ involvement from first available ^{18}FDG-PET-CT

5.1.8 Mortality

Out of our 64 patients, 11 died, representing the 17% of the sample. The Kaplan-Meier curve we obtained is presented in the *Figure 35* below.

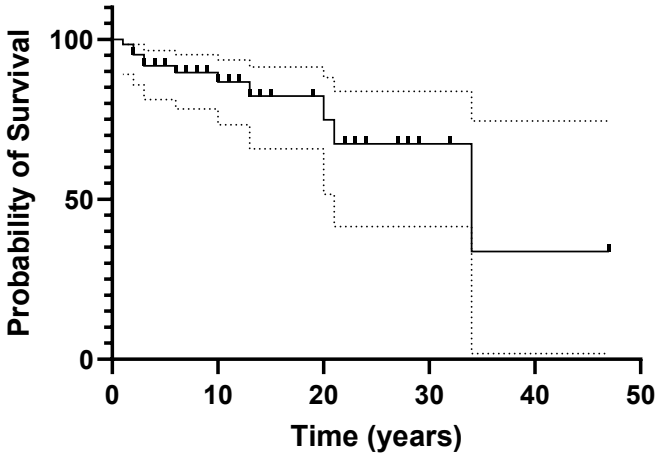


Figure 35: Kaplan-Meier curve of the sample. The independent variable is represented by the years between the diagnosis of CVID and the death or the last follow-up of the patient, while the independent variable is the probability of survival.

The causes of death are summarised in the following *Figure 36*.

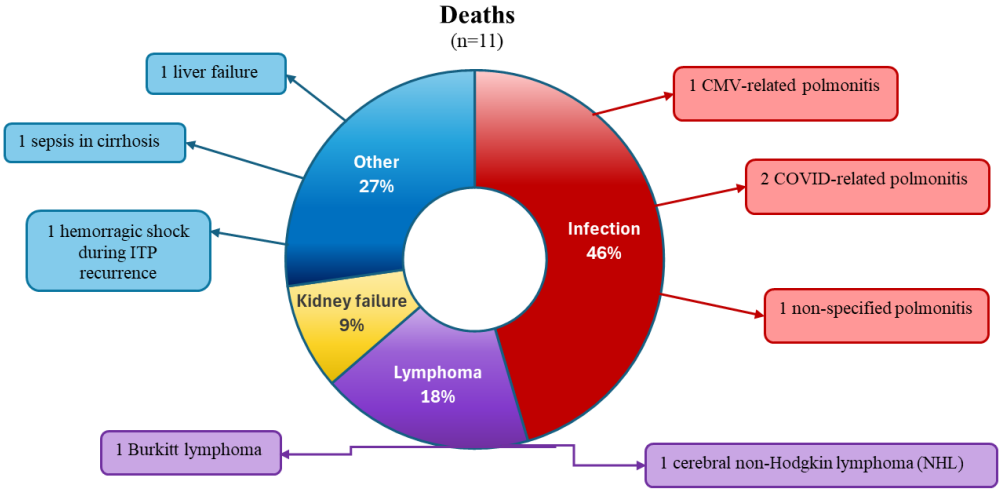


Figure 36: Causes of deaths, divided in subgroups (infection, lymphoma, kidney failure, other).

5.1.9 Treatment

All the 64 patients were under Ig replacement therapy, as aforementioned; additionally, 40 of them received a specific therapy.

The different treatment strategies were represented by:

- Steroids;
- DMARDs (mainly Azathioprine and Mycophenolate);
- Rituximab (anti-CD20), either alone or in combination with DMARDs;
- Treatments for the complications, such as R-CHOP for lymphomas.

The data are showed in the *Figure 37* below.

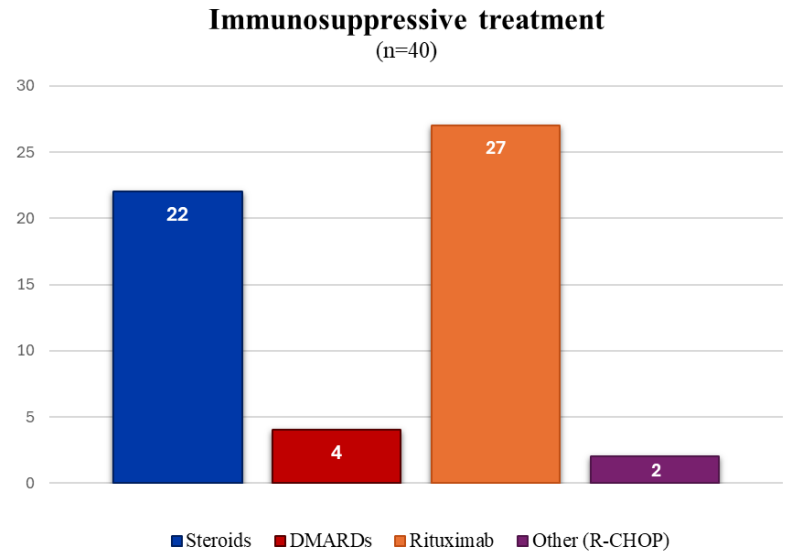


Figure 37: Treatment strategies used for our population.

The main reasons behind the treatment initiation were several: decline of lung function parameters (reduction of DLCO >10% or FVC >5%) or respiratory failure, radiological progression, extrapulmonary progression, thrombocytopenia.

Steroids were administered at a median dose of 0.5 mg/kg, usually for cycles. Rituximab was administered mainly at a dosage of 375 mg/m^2/week for 4 weeks, from 2 to 6 cycles. 14 patients who underwent Rituximab had clear available evidence of response (*e.g.* from CT, ^{18}FDG-PET-CT, PFT or from regression of symptoms).

5.2 Comparisons between case and control group

5.2.1 Demographic characteristics

The distribution of sex did reach the statistical significance when referred to the case and control groups (*see Figure 38*).

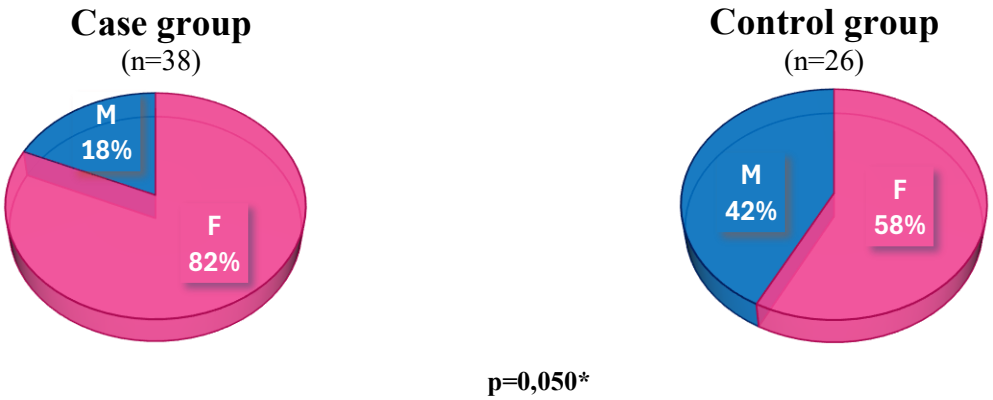


Figure 38: Sex distribution among case ("negative prognosis group") and control group ("positive prognosis group"). Using the Fisher's exact test, the analysis reached the upper limit of statistical significance (p=0,050)*

In the following *Table VI,* the demographic characteristics of the two groups are presented.

	Cases	Controls	p
Age	52.50 (43.00-67.25)	43.50 (36.50-53.00)	**0.029***
Age at first symptoms of IDCV	28.00 (13.75-36.00)	31.00 (21.25-38.25)	0.432
Age at first symptoms of GLILD	39.50 (28.50-53.00)	34.00 (31.00-47.00)	0.145
Age at diagnosis of CVID	39.00 (30.00-53.00)	35.00 (25.00-46.00)	0.222
Age at diagnosis of GLILD	47.00 (36.75-58.00)	39.00 (34.00-47.50)	0.109
Diagnostic delay of CVID	9.00 (4.00-16.00)	3.00 (0.00-6.00)	**0.002****
Diagnostic delay of GLILD	1.00 (0.00-7.00)	2.00 (0.00-8.00)	0.780

Table VI: Demographic characteristics of the sample

Median and interquartile range of age, age at diagnosis, age at first symptoms and diagnostic delay of respectively CVID and GLILD in the sample, divided between cases and controls. U Mann-Whitney test was performed to determine the p-value.

The only variables that presented a statistical significance were age (*p=0.029**) and diagnostic delay for the diagnosis of common variable immunodeficiency (*p=0.002***).

5.2.2 Biopsies and histological findings

Comparisons between cases and controls did not lead to significant differences. The results are presented in the following *Table VII*.

	Cases	Controls	p
Granuloma - Lung	8 (80.0%)	1 (20.0%)	0.089
L.F.H. - Lung	7 (77.8%)	3 (60%)	0.580
Fibrosis - Lung	3 (33.3%)	0 (0.0%)	0.229
Granuloma – Lymph node	7 (70.0%)	5 (62.5%)	1.000
L.F.H. – Lymph node	12 (92.3%)	6 (75.0%)	0.531
Fibrosis – Lymph node	1 (7.7%)	0 (0.0%)	1.000
Granuloma - Other	5 (83.3%)	4 (50.0%)	0.301
L.F.H. - Other	3 (60.0%)	5 (100.0%)	0.144
Fibrosis - Other	1 (16.7%)	1 (12.5%)	1.000

Table VII: Histological findings

Frequencies and percentages (referred to the total of the case/control group) of most common histological findings from respectively lung biopsy, lymph node biopsy and other organs' biopsy, divided between cases and controls. Fisher's exact test was performed to determine the p-value. L.F.H. = lymphoid follicular hyperplasia.

Every finding was more common in the case group, if considering lung and lymph node biopsies. Fibrosis, in particular, was absent in the control group in both types of biopsies. In any case, no statistical significance was reached.

5.2.3 Laboratory parameters

5.2.3.1 Immunoglobulin serum levels and Ig replacement therapy

The immunoglobulin serum levels (IgG, IgA, IgM) at diagnosis of cases and controls are summarized in the following *Table VIII*.

	Cases	Controls	p
IgG (mg/dL)	189.00 (79.00-320.00)	298.00 (189.50-397.80)	**0.027***
IgA (mg/dL)	8.00 (6.00-23.00)	16.50 (4.25-25.80)	0.673
IgM (mg/dL)	17.00 (8.00-56.00)	19.00 (16.00-35.50)	0.914

Table VIII: Immunoglobulin serum levels at diagnosis

Median and interquartile range of IgG, IgA and IgM, divided between case and control group. U Mann-Whitney test was performed to determine the p-value.

All the immunoglobulin levels were reduced in the case group, but the analysis reached the statistical significance only for the IgG subgroup (*p=0.027**), as it can be seen in *Figure 39*.

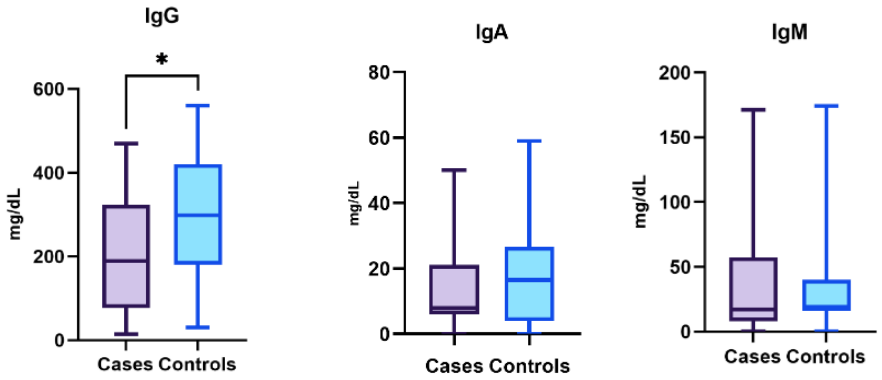


Figure 39: Comparison in IgG, IgA and IgM serum levels at diagnosis between cases and controls.

Then, IgTL levels were analysed. The data were collected at first and last available IgRT dosage (steady state). The results are summarised in the following *Table IX*.

	Cases	Controls	p
First available IgRT (mg/kg/4 weeks)	400 (340-440)	357 (303-408)	0.169
First available IgTL (mg/dL)	581 (431-710)	629 (406-769)	0.653
Last available IgRT (mg/kg/4 weeks)	421 (377-495)	377 (332-437)	**0.042***
Last available IgTL (mg/dL)	762 (600-883)	726 (660-860)	0.797

Table IX: First and last available IgRT doses and IgTL

Median and interquartile range of respectively first and last available IgRT and IgTL, divided between case and control group. U Mann-Whitney test was performed to determine the p-value.

The case group required the highest doses of IgRT and presented the lowest IgTL both at first and last observation. The levels of last available IgRT were significantly different (*p=0.042**), as shown in *Figure 40*.

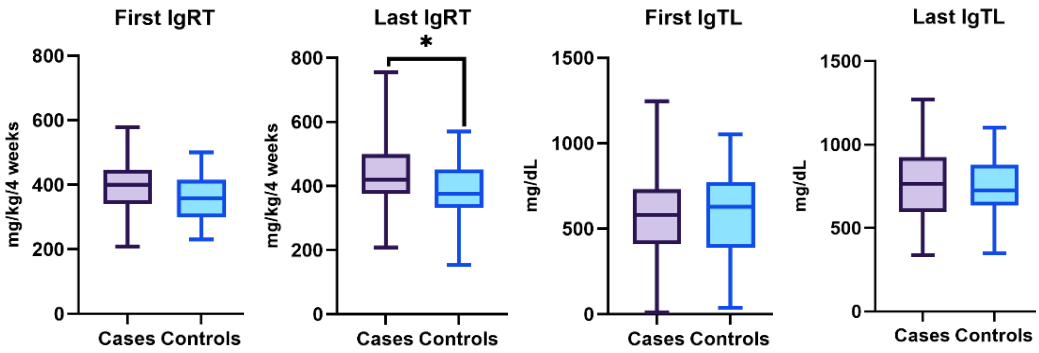


Figure 40: Comparison in IgRT doses and IgTL serum levels at diagnosis and at last available control (steady state) between cases and controls.

5.2.3.2 Analysis of serum B and T cell subpopulations

Peripheral blood leukocytes count was 4720 (3450-6500) for the case group and 5235 (3875-5800) for the control group, with no statistical significance (*p=0.951*). The median percentage of lymphocytes was 28% (22%-35.5%) for the cases and 33.55% (29.00%-38.25%) for the controls, again without showing a significance (*p=0.084*).

The results for T subgroups can be seen in the following *Table X.*

	Cases	Controls	p
CD3+%	82.50 (76.75-85.00)	73.84 (71.00-79.00)	**0.010****
CD4+%	44.00 (38.50-51.25)	47.05 (40.57-51.50)	0.483
CD8+%	33.30 (22.75-42.50)	26.00 (21.30-33.50)	0.098
CD4+/CD8+ ratio	1.10 (0.80-1.48)	1.58 (1.20-2.33)	**0.050***

Table X: T cells percentage and subpopulations

Median percentage and interquartiles of T cell subpopulations and median and interquartiles of CD4+/CD8+ ratio, divided between cases and controls. U Mann-Whitney test was performed to determine the p-value.

The total percentage of T cells (*p=0.010***) and the ratio CD4+/CD8+ (*p=0.050**) reached statistical significance.

The results of the B-cell immunophenotyping according to the EUROclass protocol are summarised in the *Table XI* below.

	Cases	Controls	p
CD19+%	7.60 (4.40-10.00)	10.00 (6.09-15.00)	**0.045***
Naïve %	80.60 (69.38-89.17)	84.00 (70.00-88.10)	0.948
Marginal zone %	6.40 (2.50-14.55)	3.60 (1.80-12.85)	0.586
Switched memory %	1.80 (1.08-2.20)	2.15 (0.85-3.28)	0.741
Transitional %	1.55 (0.60-4.85)	2.30 (1.45-3.90)	0.712
Plasmablasts %	0.80 (0.17-1.30)	1.00 (0.30-1.70)	0.469
$CD21^{low}$ %	11.55 (4.65-27.25)	17.75 (4.70-37.33)	0.485
CD3+CD57+%	12.60 (9.00-17.50)	14.50 (5.90-30.0)	0.662

Table XI: B cells percentage and subpopulations

Median percentage and interquartiles of B cells and subpopulations, divided between cases and controls. U Mann-Whitney test was performed to determine the p-value.

The only significant difference we found is the total percentage of B cells *(p=0.045*).*

5.2.3.3 Analysis of the bronchoalveolar lavage fluid (BALF)

The median cell count was 145.00/mm^3 (70.00-239.00) for cases and 177.00/mm^3 (87.00-400.00) for controls, without any statistical significance (*p=0.583*). Percentages of the main cell populations gave these results:

- Macrophages: 32.00% (7.00-61.500) for cases, 52.50% (36.25-69.50) for controls (*p=0.328*);
- Neutrophils: 33.50% (9.25-51.50) for cases, 3.00% (0.25-9.50) for controls (*p=0.026*);*
- Eosinophils: 0.00% (0.00-1.00) for cases and 0.00% (0.00-0.00) for controls (*p=0.449*);
- Lymphocytes: 20.50% (12.60-34.75) for cases and 30.00% (22.50-58.50) for controls (*p=0.247*).

Neutrophils percentage had a significant p-value (*p=0.026**).

For what it concerns lymphocytes subgroups, the available data for each patient are summarized in the following *Table XII*.

	Cases	Controls	p
CD3+%	88.00 (82.00-93.50)	94.50 (90.63-95.75)	0.083
CD4+/CD8+ ratio	1.10 (0.520-1.45)	4.13 (2.16-6.09)	**0.004****
CD19%	4.80 (1.50-8.22)	1.60 (1.20-2.00)	0.315
Switched memory %	11.30 (5.65-11.80)	13.70 (6.85-23.95)	0.507
Naïve %	11.30 (5.65-20.15)	15.30 (7.65-20.10)	1.000
Marginal zone %	2.10 (1.05-2.80)	1.60 (0.80-4.30)	1.000
CD21^{low} %	78.00 (70.5-81.50)	59.40 (51.45-70.20)	0.517
Transitional %	0.00 (0.00-0.00)	0.00 (0.00-0.10)	0.505

Table XII: BALF features

Median and interquartiles of BALF features in cases and controls. U Mann-Whitney test was performed to determine the p-value.

Only the ratio CD4+/CD8+ reached the statistical significance (*p=0.004***); interestingly, this parameter was significant also for the serum levels analysis, as seen in *Paragraph 5.2.3.2*.

5.2.4 Pulmonary function tests

The results of the comparison between the case and control group at first and last available PFT are summarised in the *Table XIII and XIV* below.

	Cases	Controls	p
FEV1 (L)	2.33 (1.98-3.04)	3.08 (2.55-3.59)	**0.004****
FEV1 %	82.00 (70.50-96.50)	91.50 (77.50-105.00)	0.206
FVC (L)	2.95 (2.34-3.74)	3.94 (3.31-4.62)	**<0.001*****
FVC %	82.00 (72.50-95.00)	97.00 (89.00-103.00)	**0.013***
TLC (L)	4.20 (3.77-5.47)	5.29 (5.21-5.39)	0.144
TLC %	80.00 (72.50-93.50)	95.00 (85.00-101.00)	**0.028***
DLCO (mL/min/mmHg)	16.20 (12.20-19.40)	22.90 (18.90-25.10)	**<0.001*****
DLCO %	70.50 (58.00-80.80)	91.00 (80.00-100.00)	**0.002****

Table XIII: Lung function parameters at first available PFR

FEV1, FVC, TLC, DLCO and percentages of predicted at first available PFR, divided between case and control group. U Mann-Whitney test was performed to determine the p-value.

	Cases	Controls	p
FEV1 (L)	2.23 (1.74-2.87)	2.75 (2.46-3.39)	**0.006****
FEV1 %	83.00 (71.00-90.00)	88.00 (78.00-101.00)	0.127
FVC (L)	2.87 (2.43-3.68)	3.76 (3.25-4.44)	**<0.001*****
FVC %	79.00 (70.00-96.00)	100.00 (83.00-103.00)	0.044
TLC (L)	4.50 (3.76-4.92)	5.24 (4.50-5.56)	0.063
TLC %	83.00 (74.00-94.00)	93.00 (81.50-102.00)	0.293
DLCO (mL/min/mmHg)	16.3 (11.1-18.4)	19.2 (15.9-27.0)	0.072
DLCO %	72.50 (58.30-86.50)	82.00 (73.00-90.00)	0.330

Table XIV: Lung function parameters at last available PFR

FEV1, FVC, TLC, DLCO and percentages of predicted at last available PFR, divided between case and control group. U Mann-Whitney test was performed to determine the p-value.

In *every* parameter considered, the case group had lower values than the control group. Moreover, the analysis reached a high level of significance for what it concerns FEV1 and FVC at both PFRs and DLCO at last PFR (respectively *p=0.004*** and *p=0.006*** for FEV1 at first and last PFR, *p<0.001**** for FVC in both first and last PFR, *p<0.001**** for DLCO at first PFR).

Moreover, we focused on the DLCO of cases and controls, as it was the only parameter that reached high significance in both absolute number and percentage of predicted. Interestingly, we observed that the case group, at both the first and last available PFT, almost reached the 5th percentile of DLCO's normal distribution. On the other hand, the controls' DLCO levels were quite far from the others, around the 40th-50th percentiles. The following *Figures 41 and 42* highlight this concept.

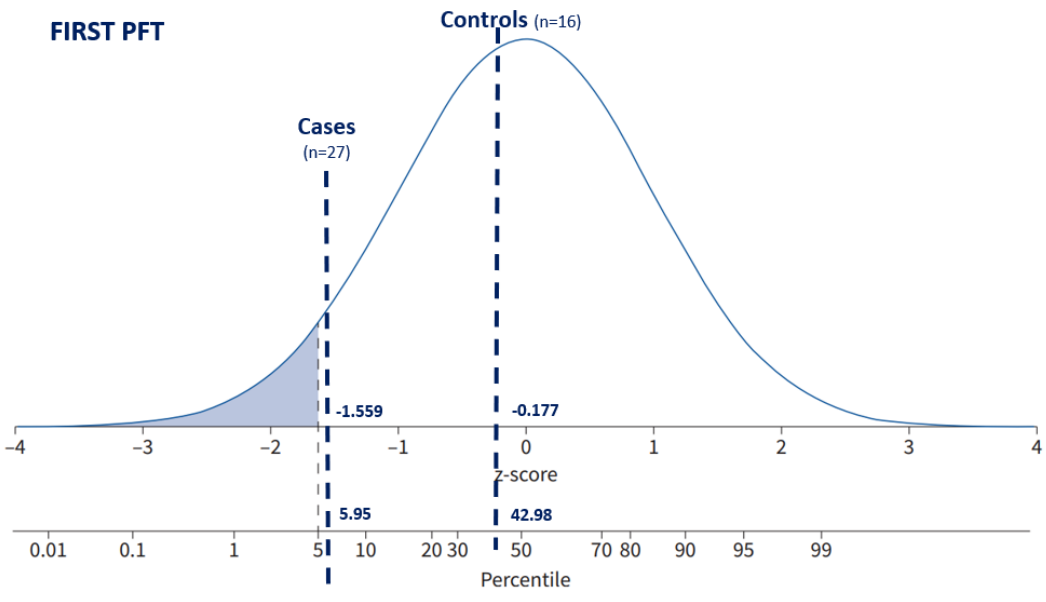


Figure 41: DLCO distribution of case and control group at first available PFT, in comparison to the normal DLCO distribution. The graph was modified from the article "ERS/ATS technical standard on interpretive strategies for routine lung function tests"[92].

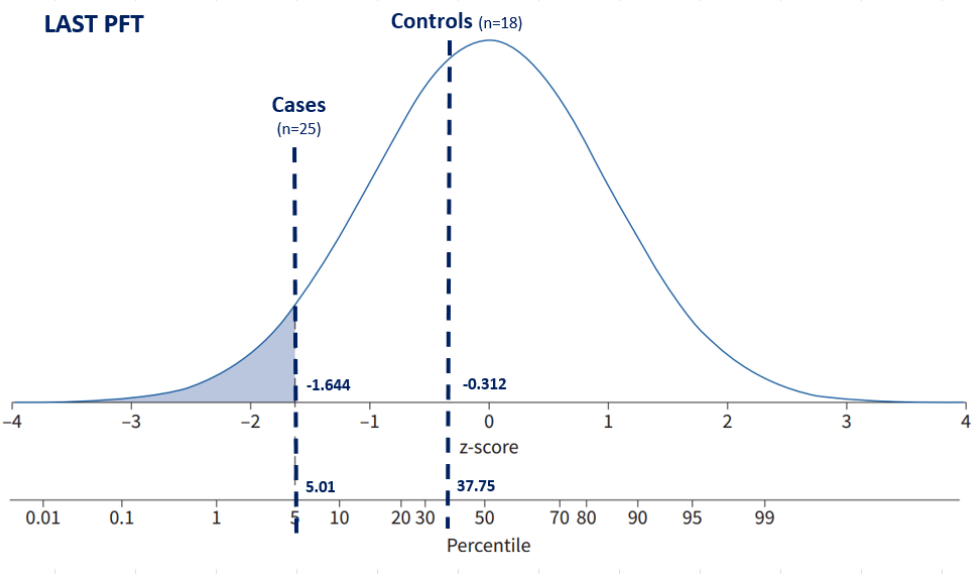


Figure 42: DLCO distribution of case and control group at last available PFT, in comparison to the normal DLCO distribution. The graph was modified from the article "ERS/ATS technical standard on interpretive strategies for routine lung function tests"[92].

5.2.5 Clinical manifestations

The analysis we conducted on clinical manifestations led to these results:

- There was no significant difference between cases and control regarding splenomegaly (*p=0.630*), nor splenectomy (*p=0.364*).
- Comparing cases and controls, no significant association was found between the two groups according to the Chapel classification. Chapel I and II were not considered, as respectively nobody and everybody was part of the category. 21 cases and 15 controls were included in Chapel III (*p=0.896*) while 3 and 3 were defined as Chapel IV (*p=0.623*).
- Autoimmune cytopenias were more present in the case group, but the difference was not significant (*p=1.000*).
- Surprisingly, organ autoimmunity was more present in the control group, but the difference did not reach the statistical significance (*p=0.175*).
- No comparison between cases and controls was conducted regarding malignancies, as lymphomas were one of the inclusion criteria for the case group.

5.2.6 CT and ^{18}FDG-PET-CT

In the following *Table XV* are summarised the main findings at first available CT.

	Cases	Controls	p
GGO	22 (71.0%)	4 (19.0%)	**<0.001*****
Nodules	26 (83.9%)	13 (61.9%)	0.105
Bronchiectasis	22 (71.0%)	6 (28.6%)	**0.004****
Lymphadenopathies	27 (87.1%)	14 (66.7%)	0.095

Table XV: Main findings at first available CT

Absolute number and percentage (referred to the total of respectively the case group or the control group) of CT findings at first available scan, divided between cases and controls. Fisher's exact test was performed to determine the p-value.

The frequence of the parameters was *always* higher in the case group than in the control group. Statistical significance was reached considering ground glass opacities (*p<0.001****) and bronchiectasis (*p=0.004***).

The main ^{18}FDG-PET-CT findings are listed in the *Table XVI* below.

	Cases	Controls	p
Supradiaphragmatic lymph nodes	22 (100.0%)	10 (90.9%)	0.333
Subdiaphragmatic lymph nodes	10 (90.9%)	18 (81.8%)	0.643
Lung	20 (90.9%)	8 (72.7%)	0.304
Spleen	12 (54.5%)	8 (72.7%)	0.456
Liver	3 (13.6%)	1 (9.1%)	1.000

Table XVI: Main findings at first available ^{18}FDG-PET-CT

Absolute number and percentage (referred to the total of respectively the case group or the control group) of ^{18}FDG-PET-CT findings at first available scan, divided between cases and controls. Fisher's exact test was performed to determine the p-value.

The frequency of involvement of almost every organ is higher in the case group than the control group, except for spleen involvement (but we already stated that it was a very common feature in the whole group). The totality of the case group presented supradiaphragmatic lymph nodes involvement. Moreover, *every* patient

had at least one extrathoracic site. Anyway, no parameter reached the statistical significance.

5.2.7 Mortality and treatment

Comparisons in mortality and immunosuppressive treatment were not analysed, as they were both inclusion criteria for the case group.

5.3 Possible prognostic markers

Further analyses were performed to enlighten if we could use some of the parameters that had a significant difference between cases and controls as prognostic markers.

Starting from the above-listed results, we considered three quantitative variables: diagnostic delay from diagnosis of CVID, IgG levels at diagnosis and DLCO% of predicted. A logistic regression analysis was performed for each variable.

The following *Figures 43, 44, 45* demonstrate the correlation between the variations of these parameters and the risk of developing a more severe condition.

The p-values for each analysis were respectively *p=0.059* for DLCO%, *p=0.050** for IgG levels, *p=0.024** for the diagnostic delay.

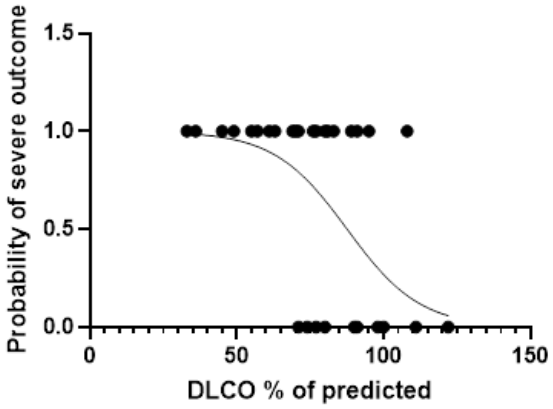


Figure 43: This graph shows that as DLCO% decreases, the probability of developing a more severe outcome increases.

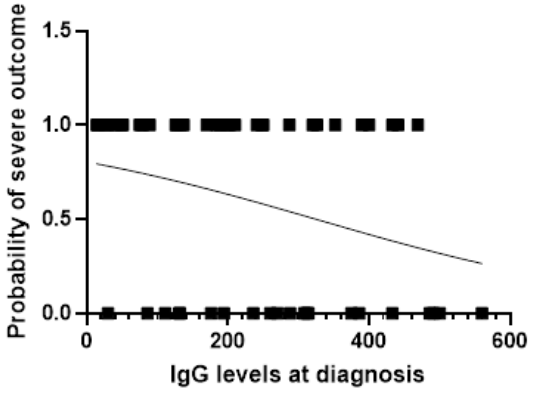


Figure 44: This graph shows that as IgG levels decreases, the probability of developing a more severe outcome increases.

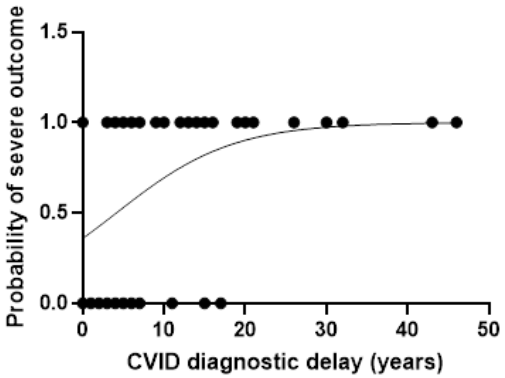


Figure 45: This graph shows that as the years of diagnostic delay of CVID rise, the probability of developing a more severe outcome increases.

The results of the multivariate analysis are shown in the *Table XVII* below.

	p	OR
DLCO% of predicted	0.090	1.007 (0.999-1.010)
IgG levels at diagnosis (mg/dL)	0.075	1.063 (0.991-1.140)
CVID diagnostic delay (years)	0.084	0.831 (0.674-1.020)

Table XVII: results of the multivariate logistic regression analysis.

p-values and Odds Ratio (with percentiles) of the multivariate regression analysis we conducted on DLCO%, IgG levels ad diagnosis and years of CVID diagnostic delay.

We calculated specificity, sensitivity and the ROC curve of a hypothetic prognostic score including these variables. We obtained a specificity of 0.952, a sensitivity of 0.750 and an Area under the curve (AUC) of 0.901, as presented in the *Figure 46* below.

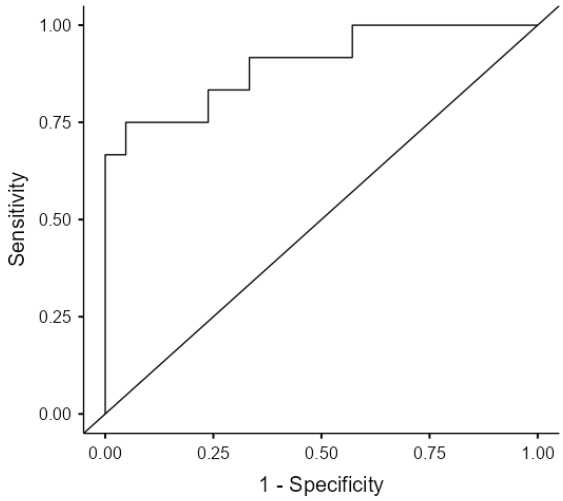


Figure 46: ROC curve of the results conducted on IgG serum levels, diagnostic delay, DLCO%.

6. Discussion

Granulomatous lymphocytic interstitial lung disease (GLILD) is a still poorly understood and relatively unknown entity that is described as an interstitial lung disease occurring in patients affected by common variable immunodeficiency (CVID), histologically associated with a lymphocytic infiltrate and/or granuloma in the lung. There is still no consensus on a common diagnostic algorithm, nor a standardized treatment approach defining when to treat patients and which drugs should be used. Moreover, so far, all the available studies are retrospective and include small cohorts of patients.

Common variable immunodeficiency is the most prevalent symptomatic primary antibody deficiency (PAD) of the adult[6] and the most diagnosed symptomatic inborn error of immunity (IEI). Rather than a single disease, its definition gathers together several disorders with or without a specific genetic background. As testified by the occurrence of GLILD, CVID is not just an "immune deficiency", but a condition where immune dysregulation may lead to infections or autoimmunity or both.

The aim of our study was to observe a multicenter GLILD cohort under different points of view, in particular clinical manifestations, mortality, radiological findings, laboratory parameters and therapeutic strategies. Then, by dividing the patients in cases and controls, we wanted to highlight putative parameters that might help distinguishing milder phenotypes from severe ones.

First of all, our data suggest that GLILD seems to preferably affect the female sex. This is not surprising, considering that females are generally more prone to develop autoimmune diseases, but there is no clear evidence about that in literature[93 94].

Secondly, we observed that GLILD could appear before CVID diagnosis. This was already mentioned in literature[48], and in our sample 28% of the enrolled patients had symptoms of GLILD as the first clinical manifestation of CVID. This supports the importance of spreading the knowledge of this peculiar ILD, since it may lead to CVID diagnosis with obvious therapeutic implications.

Thirdly, as suspected, we noticed an important diagnostic delay[9]. What is remarkable, however, is that from our data the diagnostic delay for CVID is still longer than GLILD's. This may be explained by the fact that once CVID is discovered, patients undergo screening exams to characterise the condition precisely, including pulmonary function tests and chest CT scan. Moreover, if there has already been a diagnosis of CVID, GLILD's suspicion (and presence) becomes more probable.

We also confirmed that the prognostic impact of GLILD is relevant, since we found a mortality of 17% in out cohort, that is comparable to what has been reported in the last few years: a mortality rate around 20%[47]. The causes of death were various, most of which strongly connected with GLILD: in particular, fatal infections mainly involved the already damaged patients' lungs, while lymphomas are linked to the lymphoproliferative aspect of the disease; of note, the prevalence of lymphomas in our population was 12.5%, significantly higher than what reported in the Italian CVID population (5.5%)[44]. Lastly, hepatopathy and immune cytopenia are well-known complication/co-morbidities of GLILD. Consequently, GLILD is classified as an interstitial lung disease, but our data clearly show that this is rather a lung manifestation of a wider, multisystemic condition, in line with what reported in recent literature. Indeed, we found at least one extrapulmonary involvement in 100% of cases.

Regarding extrapulmonary involvement, we then compared our data with the study by Jolles et al.[60]. The findings in common were several, including:

- Splenomegaly;
- Widespread lymphadenopathy;
- Thrombocytopenia;
- Liver disease and/or enteropathy.

Splenomegaly was very common in our sample (77%), confirming its crucial role in the diagnostic process. Lymphadenopathy was the second more common manifestation, involving 72% of the population.

Systemic involvement was almost completely represented by autoimmune cytopenias, in particular immune thrombocytopenia (ITP), which affected half the population. Autoimmune hemolytic anemia was present in 20% of the patients, being the second most frequent systemic comorbidity. Concentrating on single organ comorbidities, hepatopathy (31%) was by far the most common in our population. Thyroiditis (17%), skin involvement (11%) and enteropathy (1%) were also diagnosed in some patients.

In terms of immunologic biomarkers, we also found many similarities with what reported in other smaller cohorts (e.g. Switched memory B cells <2% and $CD21^{low}$ B cells >10%)[60].

Focusing on immunoglobulin levels, we observed a decrease in IgG, IgA and IgM serum levels of our GLILD patients. On the contrary, we did not find a significant rise of IgM, as mentioned in the literature[55]. The whole cohort was receiving immunoglobulin replacement therapy, with satisfying results. Subcutaneous administration was the preferred route of administration, as it was used by 80% of the sample; this preference was already supported by literature, as it appears to be safer and more efficient[78].

Laboratory tests gave some interesting results: the first and most important aspect is that $CD21^{low}$ B cells % was quite high in both the analyses. This increase has been already discussed widely in literature, both for its potential diagnostic and prognostic implications[63]. Several studies suggested that these cells might have a key role in autoimmunity development, as they have already been found in rheumatoid arthritis and in Sjogren's syndrome[95]. $CD21^{low}$ B cells increase is accompanied by a significant decrease in SmB cells (mainly <2%) and by an increase in large granular lymphocytes, that has already been reported by our group[63].

BALF analysis of our patients presented a more than doubled percentage of lymphocytes. Among lymphocytes, CD4/CD8 ratio of T cells was on average normal, different from what commonly found in another granulomatous ILD as sarcoidosis. B cell subpopulations varied vastly, but the key finding was the clear predominance of activated $CD21^{low}$ B cells, representing more than 75% of B

cells; this is in line with other reports on small cohorts of GLILD patients[77] and strongly supports a pathogenetic role of this subpopulation, with clear therapeutic implications. Putting together peripheral blood and BALF findings, these alterations seem to confirm the state of imbalance which characterise GLILD patients' immune system.

Genetic tests of our GLILD patients confirmed the already mentioned roles of TACI[57] and CTLA-4[50] in the pathogenesis of the disease. Unfortunately, the sample size and the incomplete coverage of genetic screening in out cohort do not allow us drawing any conclusions.

Concerning GLILD diagnosis, PFTs', radiology's and biopsies' results are aligned with what is known so far. GLILD patients have a worse lung function in comparison to general population, regarding both volumes and gas transfer [67]. CT scans confirmed that the main findings are ground-glass opacities, fibrosis, presence of nodules and lymphadenopathies[69 23 70]. Biopsies showed a prevalence of lymphocytic infiltration, granulomatous inflammation and fibrosis[75].

These results seem to agree with the most recent articles on possible predictors for GLILD. In particular, we compared the parameters we found with those suggested by several studies, and we confirmed that by combination of two clinical manifestations (splenomegaly and autoimmune cytopenia), one lung function index (DLCO%) and one immunologic variable ($CD21^{low}$ %)[63], 59 GLILD patients out of 64 would have had a correct diagnosis before histologic confirmation, representing 92% of the total cohort.

Finally, the analysis conducted on therapeutic strategies shows that rituximab is the most used treatment for GLILD patients, mainly in monotherapy, with more than half of the treated patients (52%) showing clear signs of response; the efficacy of Rituximab monotherapy has already been reported, but literature still does not support its use as first line treatment and out cohort is the biggest cohort described, ever, of GLILD patients treated with Rituximab[83 84].

Moving to the preliminary research for potential prognostic markers, we performed a case-control analysis to assess if the severity of the clinical

phenotype could be suspected before the decline of lung function parameters and the radiological progression of the interstitial lung disease.

We found that patients with more severe phenotype were older and had longer CVID diagnostic delay, suggesting that probably GLILD had more time to worsen before its diagnosis and therapy initiation. Older age also means a more remote symptoms' onset; hopefully, the ongoing and progressive reduction of the CVID diagnostic delay due to more widespread disease awareness will limit its prognostic impact.

The case group also had lower IgG serum level at CVID diagnosis and needed a higher dosage of IgRT to achieve similar, despite tendentially lower, IgTL. This further underlines the need for IgRT personalization in CVID patients, according to disease related complications. Of note, an impact of IgRT dosage on lung function decline has been reported in a small cohort[96].

Focusing on serum and BALF analyses, our data, despite preliminary, seem to suggest a relationship between the degree of immune imbalance and the severity of GLILD.

In particular, if serum B and T cells subpopulation do not seem significantly different between cases and controls, BALF analysis showed that cases presented tendentially higher $CD21^{low}$ B cells % with a significantly lower CD4/CD8 T cells ratio, within the normal range. On the other hand, the control group had a significantly increased CD4/CD8 ratio, higher than the threshold considered supportive for the diagnosis of sarcoidosis. Our data support what has already been observed in a small cohort, that a higher ratio could lead to a favourable disease course, closer to that occurring in most sarcoidosis patients[97].

CT scans confirmed what was already studied in the past years: patients with severe GLILD have significantly greater extension and severity of lung involvement at chest HRCT scan as compared to patients with stable disease[72]. Ground glass opacities and bronchiectasis appeared to be the most relevant findings in terms of prognostic impact. However, a centralized revision of lung

imaging by expert chest Radiologists is ongoing and will hopefully lead to further prognostic considerations.

Even ^{18}FDG-PET-CT scans gave important information: almost underlying a more extensive involvement in the case group, meaning that a more severe condition may be linked to a more spread diffusion of the disease.

As expected, severe patients tended to have a more critical lung function impairment in comparison to controls. Lung parameters were lower than the general population in the whole sample, but a statistical significance has been achieved in FEV1 and FVC levels when comparing mild and severe GLILD patients.

Interestingly, the comparison of DLCO at the first and last available spirometry in GLILD patients showed a difference: while at the first PFT the parameter reached high significance, at the last test it did not anymore. This might be due to the fact that also controls' lung function declined through time, altering the difference between the two groups. Another reason may be found in the effectiveness of the immunosuppressive therapy used for the cases. In any case, even at last measurement, the parameter is still very close to the significance and the DLCO value in severe patients, despite treatment, is around the 5th percentile of what expected in the general population.

Finally, trying to put together all those findings potentially related to a worse prognosis, we observed that:

- As DLCO% declines, the probability of a poorer outcome rises;
- The lower the IgG levels at diagnosis are, the higher is the risk of a more severe disease;
- A longer diagnostic delay puts the patient more at risk of having a negative prognosis.

The combination of these variables seems to provide a moderately accurate prognostic test, with an area under the curve (AUC) of 0.901. In our opinion, after validation in larger cohorts, a score based on these components may help clinicians to discriminate between who needs deeper examinations or not,

addressing the decision on prescribing further diagnostic exams or anticipating the therapeutic intervention.

6.1 Limitations of the study

The present study has different limitations, mainly linked to the small size of the sample, the retrospective nature of the study and the difficulty to organize a multicenter analysis. We gathered a lot of data, but some information were not complete and the follow-up CRF were not always updated. A few patients moved from or were referred to other hospitals, and the investigators were not always in possession of the complete medical records. Moreover, the design of the case and control group was made upon criteria we considered to be the best to define the mild and severe phenotype, but there may be some confounding factors (*e.g.* the eventual use of treatments for overlapping conditions; the fact that some data were gained indirectly from subsequent records, possibly being incomplete). Anyway, GLILD is a complication occurring in around 20% of CVID patients and CVID itself is a rare disease; thus, larger and more accurate studies are not easy to be done and the ongoing prospective studies will take years to give useful results. Finally, the heterogeneity of the disease would also make it difficult to design randomized controlled interventional trials.

7. Conclusions

Herein we reported on one of the largest cohorts of GLILD patients, showing preliminary data of a national study that will hopefully contribute to better understanding GLILD behaviour and its prognostic and therapeutic features.

We confirmed that GLILD needs to be considered as a multisystemic entity with a pulmonary focus. Consequently, lung biopsy remains an important diagnostic tool, but the approach to the condition can be more various, considering also less invasive and more "peripheric" histologic or clinical-radiologic diagnostic approaches. DLCO%, $CD21^{low}$ B cells %, splenomegaly and autoimmune cytopenias, as well al BALF cell analysis (together with microbiological differential diagnosis on BALF) represent indeed useful tools, to be combined with consistent HRCT findings, to reach a diagnostic consensus. This could not only lead to a safer process for the patient, but could also help clinicians to address the first stage of the diagnostic process without the need of prescribing invasive bioptic approach.

Moreover, we found more hints of an involvement of TACI and CTLA-4 in GLILD's pathogenesis. This, in the future, could pave the way for the utilization of new therapeutic strategies.

Focusing on therapy, we then highlighted the role that replacement therapy and Rituximab are playing in the patient management.

Finally, with the case-control analysis, we checked the impact on different pathogenic aspects of a milder or a more severe manifestation of GLILD. What we obtained is that lung function is heavily declined in patients with progressive disease, and that greater pathology is visible at CT and ^{18}FDG-PET-CT scans. This information may suggest that the rapid decline of GLILD patients' functionality could be intercepted by strictly monitoring these variables. Considering that GLILD's mortality rate is quite high, this could be useful to anticipate the treatment initiation in those patients with worse prognostic indicators. We also proposed that combining different clinical, laboratory and functional parameters (*e.g.* serum IgG levels, diagnostic delay of CVID and

DLCO %) a prognostic score might be designed, to be validated in prospective cohorts, that might be definitely helpful for clinicians.

In conclusion, this study has considered many aspects of GLILD, but further studies are needed, possibly with larger cohorts and a prospective design. The hope and goal for the future needs to be a more standardized vision of the disease, with a globally accepted diagnostic process and a common management, together with a personalized approach when treatment is needed.

8. References

[1] Bousfiha A, Jeddane L, Picard C, Ailal F, Bobby Gaspar H, Al-Herz W, Chatila T, Crow YJ, Cunningham-Rundles C, Etzioni A, Franco JL, Holland SM, Klein C, Morio T, Ochs HD, Oksenhendler E, Puck J, Tang MLK, Tangye SG, Torgerson TR, Casanova JL, Sullivan KE. The 2017 IUIS Phenotypic Classification for Primary Immunodeficiencies. J Clin Immunol. 2018 Jan;38(1):129-143. doi: 10.1007/s10875-017-0465-8. Epub 2017 Dec 11. PMID: 29226301; PMCID: PMC5742599.

[2] Bousfiha A, Moundir A, Tangye SG, Picard C, Jeddane L, Al-Herz W, Rundles CC, Franco JL, Holland SM, Klein C, Morio T, Oksenhendler E, Puel A, Puck J, Seppänen MRJ, Somech R, Su HC, Sullivan KE, Torgerson TR, Meyts I. The 2022 Update of IUIS Phenotypical Classification for Human Inborn Errors of Immunity. J Clin Immunol. 2022 Oct;42(7):1508-1520. doi: 10.1007/s10875-022-01352-z. Epub 2022 Oct 6. PMID: 36198931.

[3] Kobrynski L, Powell RW, Bowen S. Prevalence and morbidity of primary immunodeficiency diseases, United States 2001–2007. J Clin Immunol 2014; 34: 954–961.

[4] Quinti I, Agostini C, Tabolli S, Brunetti G, Cinetto F, Pecoraro A, Spadaro G. Malignancies are the major cause of death in patients with adult onset common variable immunodeficiency. Blood. 2012 Aug 30;120(9):1953-4. doi: 10.1182/blood-2012-05-431064. PMID: 22936739.

[5]Azizi G, Rezaei N, Kiaee F, et al. T-cell abnormalities in common variable immunodeficiency. J Investig Allergol Clin Immunol 2016; 26: 233–243.

[6] Bonilla FA, Barlan I, Chapel H, et al. International Consensus Document (ICON): Common Variable Immunodeficiency Disorders. J Allergy Clin Immunol Pract. 2016;4(1):38-59. doi: 10.1016/j.jaip.2015.07.025.

[7] ESID Registry Working Party. ESID Registry - Working definitions for clinical diagnosis of IEI. 2019;(Cid):1-33. https://esid.org/content/download/16792/456144/file/ESIDRegistry_ClinicalCriteria.pdf

[8] Stray-Pedersen A, AT, Frøland SS. Primary immunodeficiency diseases in Norway. J Clin Immunol. 2000;20:477–485.

[9] Odnoletkova I, Kindle G, Quinti I, Grimbacher B, Knerr V, Gathmann B, Ehl S, Mahlaoui N, Van Wilder P, Bogaerts K, de Vries E; Plasma Protein Therapeutics Association (PPTA) Taskforce. The burden of common variable immunodeficiency disorders: a retrospective analysis of the European Society for Immunodeficiency (ESID) registry data. Orphanet J Rare Dis. 2018 Nov 12;13(1):201. doi: 10.1186/s13023-018-0941-0. PMID: 30419968; PMCID: PMC6233554.

[10] Chapel H, Lucas M, Lee M, Bjorkander J, Webster D, Grimbacher B, Fieschi C, Thon V, Abedi MR, Hammarstrom L. Common variable immunodeficiency disorders: division into distinct clinical phenotypes. Blood. 2008 Jul 15;112(2):277-86. doi: 10.1182/blood-2007-11-124545. Epub 2008 Mar 4. PMID: 18319398.

[11] Modified from: Bang TJ, Richards JC, Olson AL, Groshong SD, Gelfand EW, Lynch DA. Pulmonary Manifestations of Common Variable Immunodeficiency. J Thorac Imaging. 2018 Nov;33(6):377-383. doi: 10.1097/RTI.0000000000000350. PMID: 30067570.

[12] Chapel H, Lucas M, Patel S, et al. Confirmation and improvement of criteria for clinical phenotyping in common variable immunodeficiency disorders in replicate cohorts. J Allergy Clin Immunol. 2012;130(5):1197- 1198.e9. doi:10.1016/j.jaci.2012.05.046.

[13] Wehr C, Kivioja T, Schmitt C, et al. The EUROclass trial: Defining subgroups in common variable immunodeficiency. Blood. 2008;111(1):77-85. doi:10.1182/blood-2007-06-091744.

[14] Warnatz K, Denz A, Dräger R, Braun M, Groth C, Wolff-Vorbeck G, Eibel H, Schlesier M, Peter HH. Severe deficiency of switched memory B cells (CD27(+)IgM(-)IgD(-)) in subgroups of patients with common variable immunodeficiency: a new approach to classify a heterogeneous disease. Blood. 2002 Mar 1;99(5):1544-51. doi: 10.1182/blood.v99.5.1544. PMID: 11861266.

[15] Piqueras B, Lavenu-Bombled C, Galicier L, Bergeron-van der Cruyssen F, Mouthon L, Chevret S, Debré P, Schmitt C, Oksenhendler E. Common variable immunodeficiency patient classification based on impaired B cell memory differentiation correlates with clinical aspects. J Clin Immunol. 2003 Sep;23(5):385-400. doi: 10.1023/a:1025373601374. PMID: 14601647.

[16] Chapel H, Cunningham-Rundles C. Update in understanding common variable immunodeficiency disorders (CVIDs) and the management of patients with these conditions. Br J Haematol. 2009 Jun;145(6):709-27. doi: 10.1111/j.1365-2141.2009.07669.x. Epub 2009 Mar 30. PMID: 19344423; PMCID: PMC2718064.

[17] Katz U, Achiron A, Sherer Y, Shoenfeld Y. Safety of intravenous immunoglobulin (IVIG) therapy. Autoimmun Rev. 2007 Mar;6(4):257-9. doi: 10.1016/j.autrev.2006.08.011. Epub 2006 Aug 28. PMID: 17317619.

[18] Jolles S, Rojavin MA, Lawo JP, Nelson R Jr, Wasserman RL, Borte M, Tortorici MA, Imai K, Kanegane H. Long-Term Efficacy and Safety of Hizentra® in Patients with Primary Immunodeficiency in Japan, Europe, and the United States: a Review of 7 Phase 3 Trials. J Clin Immunol. 2018 Nov;38(8):864-875. doi: 10.1007/s10875-018-0560-5. Epub 2018 Nov 10. PMID: 30415311; PMCID: PMC6292970.

[19] https://info4pi.org/library/educational-materials/10-warning-signs

[20] Cinetto F, Scarpa R, Rattazzi M, Agostini C. The broad spectrum of lung diseases in primary antibody deficiencies. Eur Respir Rev. 2018 Aug 29;27(149):180019. doi: 10.1183/16000617.0019-2018. PMID: 30158276; PMCID: PMC9488739.

[21] Peckham D, Scambler T, Savic S, et al. The burgeoning field of innate immune-mediated disease and autoinflammation. J Pathol 2017; 241: 123–139.

[22] Baumann U, Routes JM, Soler-Palacín P, Jolles S. The Lung in Primary Immunodeficiencies: New Concepts in Infection and Inflammation. Front Immunol. 2018 Aug 8;9:1837. doi: 10.3389/fimmu.2018.01837. PMID: 30147696; PMCID: PMC6096054.

[23] Hampson FA, Chandra A, Screaton NJ, Condliffe A, Kumararatne DS, Exley AR, Babar JL. Respiratory disease in common variable immunodeficiency and other primary immunodeficiency disorders. Clin Radiol. 2012 Jun;67(6):587-95. doi: 10.1016/j.crad.2011.10.028. Epub 2012 Jan 9. PMID: 22226567.

[24] Kainulainen L, Vuorinen T, Rantakokko-Jalava K, Osterback R, Ruuskanen O. Recurrent and persistent respiratory tract viral infections in patients with primary hypogammaglobulinemia. J Allergy Clin Immunol. 2010 Jul;126(1):120-6. doi: 10.1016/j.jaci.2010.04.016. Epub 2010 Jun 11. PMID: 20541246; PMCID: PMC7112312.

[25] Patuzzo G, Barbieri A, Tinazzi E, Veneri D, Argentino G, Moretta F, Puccetti A, Lunardi C. Autoimmunity and infection in common variable immunodeficiency (CVID). Autoimmun Rev. 2016 Sep;15(9):877-82. doi: 10.1016/j.autrev.2016.07.011. Epub 2016 Jul 6. PMID: 27392505.

[26] Sperlich JM, Grimbacher B, Workman S, Haque T, Seneviratne SL, Burns SO, Reiser V, Vach W, Hurst JR, Lowe DM. Respiratory Infections and Antibiotic Usage in Common Variable Immunodeficiency. J Allergy Clin Immunol Pract. 2018 Jan-Feb;6(1):159-168.e3. doi: 10.1016/j.jaip.2017.05.024. Epub 2017 Jul 19. PMID: 28734862; PMCID: PMC7185402.

[27] Hurst JR, Workman S, Garcha DS, et al. Activity, severity and impact of respiratory disease in primary antibody deficiency syndromes. J Clin Immunol 2014; 34: 68–75.

[28] Sperlich JM, Grimbacher B, Workman S, et al. Respiratory infections and antibiotic usage in common variable immunodeficiency. J Allergy Clin Immunol Pract 2018; 6: 159–168.

[29] Odnoletkova I, Kindle G, Quinti I, et al. The burden of common variable immunodeficiency disorders: a retrospective analysis of the European Society for Immunodeficiency (ESID) registry data. Orphanet J Rare Dis. 2018 Nov 12;13(1):201.

[30] Berger M, Geng B, Cameron DW, Murphy LM, Schulman ES. Primary immune deficiency diseases as unrecognized causes of chronic respiratory disease. Respir Med. 2017 Nov;132:181-188. doi: 10.1016/j.rmed.2017.10.016. Epub 2017 Oct 21. PMID: 29229095.

[31] McCullagh BN, Comellas AP, Ballas ZK, et al. Antibody deficiency in patients with frequent exacerbations of chronic obstructive pulmonary disease (COPD). PLoS One 2017; 12: e0172437.

[32] Bondioni MP, Soresina A, Lougaris V, Gatta D, Plebani A, Maroldi R. Common variable immunodeficiency: computed tomography evaluation of bronchopulmonary changes including nodular lesions in 40 patients. Correlation with clinical and immunological data. J Comput Assist Tomogr. 2010 May-Jun;34(3):395-401. doi: 10.1097/RCT.0b013e3181cad9da. PMID: 20498543.

[33] Gregersen S, Aaløkken TM, Mynarek G, Kongerud J, Aukrust P, Frøland SS, Johansen B. High resolution computed tomography and pulmonary function in common variable immunodeficiency. Respir Med. 2009 Jun;103(6):873-80. doi: 10.1016/j.rmed.2008.12.015. Epub 2009 Feb 1. PMID: 19181508.

[34] Touw CM, van de Ven AA, de Jong PA, Terheggen-Lagro S, Beek E, Sanders EA, van Montfrans JM. Detection of pulmonary complications in common variable immunodeficiency. Pediatr Allergy Immunol. 2010 Aug;21(5):793-805. doi: 10.1111/j.1399-3038.2009.00963.x. Epub 2009 Nov 13. PMID: 19912551.

[35] Martínez García MA, de Rojas MD, Nauffal Manzur MD, Muñoz Pamplona MP, Compte Torrero L, Macián V, Perpiñá Tordera M. Respiratory disorders in common variable immunodeficiency. Respir Med. 2001 Mar;95(3):191-5. doi: 10.1053/rmed.2000.1020. PMID: 11266236.

[36] Curtin JJ, Webster AD, Farrant J, Katz D. Bronchiectasis in hypogammaglobulinaemia--a computed tomography assessment. Clin Radiol. 1991 Aug;44(2):82-4. doi: 10.1016/s0009-9260(05)80501-x. PMID: 1884591.

[37] Quinti I, Soresina A, Spadaro G, Martino S, Donnanno S, Agostini C, Claudio P, Franco D, Maria Pesce A, Borghese F, Guerra A, Rondelli R, Plebani A; Italian Primary Immunodeficiency Network. Long-term follow-up and outcome of a large cohort of patients with common variable immunodeficiency. J Clin Immunol. 2007 May;27(3):308-16. doi: 10.1007/s10875-007-9075-1. Epub 2007 Feb 14. PMID: 17510807.

[38] Correa-Jimenez O, Restrepo-Gualteros S, Nino G, Cunningham-Rundles C, Sullivan KE, Fuleihan RL, Gutierrez MJ. Respiratory Comorbidities Associated with Bronchiectasis in Patients with Common Variable Immunodeficiency in the USIDNET Registry. J Clin Immunol. 2023 Nov;43(8):2208-2220. doi: 10.1007/s10875-023-01593-6. Epub 2023 Nov 7. PMID: 37932514.

[39] Tanaka N, Kim JS, Bates CA, Brown KK, Cool CD, Newell JD, Lynch DA. Lung diseases in patients with common variable immunodeficiency: chest radiographic, and computed tomographic findings. J Comput Assist Tomogr. 2006 Sep-Oct;30(5):828-38. doi: 10.1097/01.rct.0000228163.08968.26. PMID: 16954938.

[40] Bang TJ, Richards JC, Olson AL, Groshong SD, Gelfand EW, Lynch DA. Pulmonary Manifestations of Common Variable Immunodeficiency. J Thorac Imaging. 2018 Nov;33(6):377-383. doi: 10.1097/RTI.0000000000000350. PMID: 30067570.

[41] Gregersen S, Aaløkken TM, Mynarek G, Fevang B, Holm AM, Ueland T, Aukrust P, Kongerud J, Johansen B, Frøland SS. Development of pulmonary abnormalities in patients with common variable immunodeficiency: associations with clinical and immunologic factors. Ann Allergy Asthma Immunol. 2010 Jun;104(6):503-10. doi: 10.1016/j.anai.2010.04.015. PMID: 20568383.

[42] Aghamohammadi A, Parvaneh N, Tirgari F, Mahjoob F, Movahedi M, Gharagozlou M, Mansouri M, Kouhi A, Rezaei N, Webster D. Lymphoma of mucosa-associated lymphoid tissue in common variable immunodeficiency. Leuk Lymphoma. 2006 Feb;47(2):343-6. doi: 10.1080/10428190500285285. PMID: 16321869.

[43] Reichenberger F, Wyser C, Gonon M, Cathomas G, Tamm M. Pulmonary mucosa-associated lymphoid tissue lymphoma in a patient with common variable immunodeficiency syndrome. Respiration. 2001;68(1):109-12. doi: 10.1159/000050475. PMID: 11223743.

[44] Pulvirenti F, Pecoraro A, Cinetto F, Milito C, Valente M, Santangeli E, Crescenzi L, Rizzo F, Tabolli S, Spadaro G, Agostini C, Quinti I. Gastric Cancer Is the Leading Cause of Death in Italian Adult Patients With Common Variable Immunodeficiency. Front Immunol. 2018 Nov 5;9:2546. doi: 10.3389/fimmu.2018.02546. PMID: 30455695; PMCID: PMC6230622.

[45] Tafuro F, Corradi M. An approach to interpreting restrictive spirometric pattern results in occupational settings. Med Lav 2016; 107: 419–436.

[46] Fernández Pérez ER. Granulomatous lymphocytic interstitial lung disease. Immunol Allergy Clin North Am. 2012 Nov;32(4):621-32. doi: 10.1016/j.iac.2012.08.003. Epub 2012 Sep 12. PMID: 23102069.

[47] Hurst JR, Verma N, Lowe D, Baxendale HE, Jolles S, Kelleher P, Longhurst HJ, Patel SY, Renzoni EA, Sander CR, Avery GR, Babar JL, Buckland MS, Burns S, Egner W, Gompels MM, Gordins P, Haddock JA, Hart SP, Hayman GR, Herriot R, Hoyles RK, Huissoon AP, Jacob J, Nicholson AG, Rassl DM, Sargur RB, Savic S, Seneviratne SL, Sheaff M, Vaitla PM, Walters GI, Whitehouse JL, Wright PA, Condliffe AM. British Lung Foundation/United Kingdom Primary Immunodeficiency Network Consensus Statement on the Definition, Diagnosis, and Management of Granulomatous-Lymphocytic Interstitial Lung Disease in Common Variable Immunodeficiency Disorders. J Allergy Clin Immunol Pract. 2017 Jul-Aug;5(4):938-945. doi: 10.1016/j.jaip.2017.01.021. Epub 2017 Mar 25. PMID: 28351785.

[48] Tashtoush B, Memarpour R, Ramirez J, Bejarano P, Mehta J. Granulomatous-lymphocytic interstitial lung disease as the first manifestation of common variable immunodeficiency. Clin Respir J. 2018. doi:10.1111/crj.12511.

[49] Bates CA, Ellison MC, Lynch DA, Cool CD, Brown KK, Routes JM. Granulomatous-lymphocytic lung disease shortens survival in common variable immunodeficiency. J Allergy Clin Immunol. 2004 Aug;114(2):415-21. doi: 10.1016/j.jaci.2004.05.057. PMID: 15316526.

[50] Kuehn HS, Ouyang W, Lo B et al. Immune dysregulation in human subjects with heterozygous germline mutations in CTLA4. Science 2014;345:1623-1627 PMID 25213377 PMC4371526.

[51] Fraz MSA, Michelsen AE, Moe N, Aaløkken TM, Macpherson ME, Nordøy I, Aukrust P, Taraldsrud E, Holm AM, Ueland T, Jørgensen SF, Fevang B. Raised Serum Markers of T Cell Activation and Exhaustion in Granulomatous-Lymphocytic Interstitial Lung Disease in Common Variable Immunodeficiency. J Clin Immunol. 2022 Oct;42(7):1553-1563. doi: 10.1007/s10875-022-01318-1. Epub 2022 Jul 5. PMID: 35789314; PMCID: PMC9255534.

[52] Lui VG, Ghosh T, Rymaszewski A, Chen S, Baxter RM, Kong DS, Ghosh D, Routes JM, Verbsky JW, Hsieh EWY. Dysregulated Lymphocyte Antigen Receptor Signaling in Common Variable Immunodeficiency with Granulomatous Lymphocytic Interstitial Lung Disease. J Clin Immunol. 2023 Aug;43(6):1311-1325. doi: 10.1007/s10875-023-01485-9. Epub 2023 Apr 24. PMID: 37093407; PMCID: PMC10524976.

[53] Matson EM, Abyazi ML, Bell KA, Hayes KM, Maglione PJ. B Cell Dysregulation in Common Variable Immunodeficiency Interstitial Lung Disease. Front Immunol. 2021 Feb 5;11:622114. doi: 10.3389/fimmu.2020.622114. PMID: 33613556; PMCID: PMC7892472.

[54] Durandy A, Kracker S, Fischer A. Primary antibody deficiencies. Nat Rev Immunol. 2013;13(7):519-533. doi:10.1038/nri3466.

[55] Maglione PJ, Gyimesi G, Cols M, et al. BAFF-driven B cell hyperplasia underlies lung disease in common variable immunodeficiency. JCI insight. 2019;4(5):1-18. doi:10.1172/jci.insight.122728.

[56] Varzaneh FN, Keller B, Unger S, Aghamohammadi A, Warnatz K, Rezaei N. Cytokines in common variable immunodeficiency as signs of immune dysregulation and potential therapeutic targets - A review of the current knowledge. J Clin Immunol. 2014;34(5):524-543. doi:10.1007/s10875-014-0053-0.

[57] Zhang Y, Li J, Zhang YM, Zhang XM, Tao J. Effect of TACI signaling on humoral immunity and autoimmune diseases. J Immunol Res. 2015;2015(Cvid). doi:10.1155/2015/247426.

[58] Linke M, Pham HTT, Katholnig K, et al. Chronic signaling via the metabolic checkpoint kinase mTORC1 induces macrophage granuloma formation and marks sarcoidosis progression. Nat Immunol. 2017;18(3):293-302. doi:10.1038/ni.3655.

[59] Deyà-Martínez A, Esteve-Solé A, Vélez-Tirado N, et al. Sirolimus as an alternative treatment in patients with granulomatous-lymphocytic lung disease and humoral immunodeficiency with impaired regulatory T cells. Pediatr Allergy Immunol. 2018. doi:10.1111/pai.12890.

[60] Jolles S, Carne E, Brouns M, El-Shanawany T, Williams P, Marshall C, Fielding P. FDG PET-CT imaging of therapeutic response in granulomatous lymphocytic interstitial lung

disease (GLILD) in common variable immunodeficiency (CVID). Clin Exp Immunol. 2017 Jan;187(1):138-145. doi: 10.1111/cei.12856. Epub 2016 Nov 28. PMID: 27896807; PMCID: PMC5167039.

[61] Mannina A, Chung JH, Swigris JJ, Solomon JJ, Huie TJ, Yunt ZX, Truong TQ, Brown KK, Achcar RD, Olson AL, Cox CW, Kligerman SJ, Curran-Everett D, Fernández Pérez ER. Clinical Predictors of a Diagnosis of Common Variable Immunodeficiency-related Granulomatous-Lymphocytic Interstitial Lung Disease. Ann Am Thorac Soc. 2016 Jul;13(7):1042-9. doi: 10.1513/AnnalsATS.201511-728OC. PMID: 27064856.

[62] Hartono S, Motosue MS, Khan S, Rodriguez V, Iyer VN, Divekar R, Joshi AY. Predictors of granulomatous lymphocytic interstitial lung disease in common variable immunodeficiency. Ann Allergy Asthma Immunol. 2017 May;118(5):614-620. doi: 10.1016/j.anai.2017.01.004. Epub 2017 Feb 20. PMID: 28254202.

[63] Cinetto F, Scarpa R, Carrabba M, Firinu D, Lougaris V, Buso H, Garzi G, Gianese S, Soccodato V, Punziano A, Lagnese G, Tessarin G, Costanzo G, Landini N, Vio S, Bondioni MP, Consonni D, Marasco C, Del Giacco S, Rattazzi M, Vacca A, Plebani A, Fabio G, Spadaro G, Agostini C, Quinti I, Milito C. Granulomatous Lymphocytic Interstitial Lung Disease (GLILD) in Common Variable Immunodeficiency (CVID): A Multicenter Retrospective Study of Patients From Italian PID Referral Centers. Front Immunol. 2021 Mar 10;12:627423. doi: 10.3389/fimmu.2021.627423. PMID: 33777011; PMCID: PMC7987811.

[64] Cabanero-Navalon MD, Garcia-Bustos V, Forero-Naranjo LF, Baettig-Arriagada EJ, Núñez-Beltrán M, Cañada-Martínez AJ, Forner Giner MJ, Catalán-Cáceres N, Martínez Francés M, Moral Moral P. Integrating Clinics, Laboratory, and Imaging for the Diagnosis of Common Variable Immunodeficiency-Related Granulomatous-Lymphocytic Interstitial Lung Disease. Front Immunol. 2022 Feb 23;13:813491. doi: 10.3389/fimmu.2022.813491. PMID: 35281075; PMCID: PMC8906473.

[65] Meerburg JJ, Hartmann IJC, Goldacker S, Baumann U, Uhlmann A, Andrinopoulou ER, Kemner V/D Corput MPC, Warnatz K, Tiddens HAWM. Analysis of Granulomatous Lymphocytic Interstitial Lung Disease Using Two Scoring Systems for Computed Tomography Scans-A Retrospective Cohort Study. Front Immunol. 2020 Oct 30;11:589148. doi: 10.3389/fimmu.2020.589148. PMID: 33193417; PMCID: PMC7662109.

[66] Cinetto F, Scarpa R, Pulvirenti F, Quinti I, Agostini C, Milito C. Appropriate lung management in patients with primary antibody deficiencies. Expert Rev Respir Med. 2019;13(9):823-838. doi:10.1080/17476348.2019.1641085.

[67] Bintalib HM, van de Ven A, Jacob J, Davidsen JR, Fevang B, Hanitsch LG, Malphettes M, van Montfrans J, Maglione PJ, Milito C, Routes J, Warnatz K, Hurst JR. Diagnostic testing for interstitial lung disease in common variable immunodeficiency: a systematic review. Front Immunol. 2023 May 8;14:1190235. doi: 10.3389/fimmu.2023.1190235. PMID: 37223103; PMCID: PMC10200864.

[68] Thickett KM, Kumararatne DS, Banerjee AK, Dudley R, Stableforth DE. Common variable immune deficiency: respiratory manifestations, pulmonary function and high-resolution CT scan findings. QJM. 2002 Oct;95(10):655-62. doi: 10.1093/qjmed/95.10.655. PMID: 12324637.

[69] Torigian DA, LaRosa DF, Levinson AI, Litzky LA, Miller WT. Granulomatous-lymphocytic interstitial lung disease associated with common variable immunodeficiency: CT findings. J Thorac Imaging. 2008;23(3):162-169. doi:10.1097/RTI.0b013e318166d32f.

[70] Scarpa R, Cinetto F, Milito C, Gianese S, Soccodato V, Buso H, Garzi G, Carrabba M, Messina E, Panebianco V, Catalano C, Morana G, Lougaris V, Landini N, Bondioni MP. Common and Uncommon CT Findings in CVID-Related GL-ILD: Correlations with Clinical Parameters, Therapeutic Decisions and Potential Implications in the Differential Diagnosis. J Clin Immunol. 2023 Nov;43(8):1903-1915. doi: 10.1007/s10875-023-01552-1. Epub 2023 Aug 7. PMID: 37548814; PMCID: PMC10661728.

[71] Buso H, Discardi C, Bez P, Muscianisi F, Ceccato J, Milito C, Firinu D, Landini N, Jones MG, Felice C, Rattazzi M, Scarpa R, Cinetto F. Sarcoidosis versus Granulomatous and Lymphocytic Interstitial Lung Disease in Common Variable Immunodeficiency: A Comparative Review. Biomedicines. 2024 Jul 6;12(7):1503. doi: 10.3390/biomedicines12071503. PMID: 39062076; PMCID: PMC11275071.

[72] Fraz MSA, Moe N, Revheim ME, Stavrinou ML, Durheim MT, Nordøy I, Macpherson ME, Aukrust P, Jørgensen SF, Aaløkken TM, Fevang B. Granulomatous-Lymphocytic Interstitial Lung Disease in Common Variable Immunodeficiency-Features of CT and 18F-FDG Positron Emission Tomography/CT in Clinically Progressive Disease. Front Immunol. 2021 Jan 26;11:617985. doi: 10.3389/fimmu.2020.617985. PMID: 33584710; PMCID: PMC7874137.

[73] Hutchinson JP, Fogarty AW, McKeever TM, Hubbard RB. In-Hospital Mortality after Surgical Lung Biopsy for Interstitial Lung Disease in the United States. 2000 to 2011. Am J Respir Crit Care Med. 2016;193(10):1161-1167. doi:10.1164/rccm.201508-1632OC.

[74] Rotolo N, Imperatori A, Dominioni L, et al. Efficacy and safety of surgical lung biopsy for interstitial disease. Experience of 161 consecutive patients from a single institution in Italy. Sarcoidosis Vasc Diffus Lung Dis. 2015.

[75] Rao N, Mackinnon AC, Routes JM. Granulomatous and lymphocytic interstitial lung disease: a spectrum of pulmonary histopathologic lesions in common variable immunodeficiency--histologic and immunohistochemical analyses of 16 cases. Hum Pathol. 2015 Sep;46(9):1306-14. doi: 10.1016/j.humpath.2015.05.011. Epub 2015 Jun 1. PMID: 26138782; PMCID: PMC4554947.

[76] Unger S, Seidl M, Schmitt-Graeff A, Böhm J, Schrenk K, Wehr C, Goldacker S, Dräger R, Gärtner BC, Fisch P, Werner M, Warnatz K. Ill-defined germinal centers and severely reduced plasma cells are histological hallmarks of lymphadenopathy in patients with

common variable immunodeficiency. J Clin Immunol. 2014 Aug;34(6):615-26. doi: 10.1007/s10875-014-0052-1. Epub 2014 May 2. PMID: 24789743.

[77] Friedmann D, Unger S, Keller B, Rakhmanov M, Goldacker S, Zissel G, Frye BC, Schupp JC, Prasse A, Warnatz K. Bronchoalveolar Lavage Fluid Reflects a T_H1-$CD21^{low}$ B-Cell Interaction in CVID-Related Interstitial Lung Disease. Front Immunol. 2021 Feb 5;11:616832. doi: 10.3389/fimmu.2020.616832. PMID: 33613543; PMCID: PMC7892466.

[78] Jolles S, Rojavin MA, Lawo JP, et al. Long-Term Efficacy and Safety of Hizentra® in Patients with Primary Immunodeficiency in Japan, Europe, and the United States: a Review of 7 Phase 3 Trials. J Clin Immunol. 2018;38(8):864-875. doi:10.1007/s10875-018-0560-5.

[79] Berger M. Subcutaneous immunoglobulin replacement in primary immunodeficiencies. Clin Immunol. 2004;112(1):1-7. doi:10.1016/j.clim.2004.02.002

[80] Arish N, Eldor R, Fellig Y, Bogot N, Laxer U, Izhar U, Rokach A. Lymphocytic interstitial pneumonia associated with common variable immunodeficiency resolved with intravenous immunoglobulins. Thorax. 2006 Dec;61(12):1096-7. doi: 10.1136/thx.2004.029819. PMID: 17114373; PMCID: PMC2117067.

[81] Boursiquot JN, Gérard L, Malphettes M, Fieschi C, Galicier L, Boutboul D, Borie R, Viallard JF, Soulas-Sprauel P, Berezne A, Jaccard A, Hachulla E, Haroche J, Schleinitz N, Têtu L, Oksenhendler E; DEFI study group. Granulomatous disease in CVID: retrospective analysis of clinical characteristics and treatment efficacy in a cohort of 59 patients. J Clin Immunol. 2013 Jan;33(1):84-95. doi: 10.1007/s10875-012-9778-9. Epub 2012 Sep 18. PMID: 22986767.

[82] Bintalib HM, Lowe DM, Mancuso G, Gkrepi G, Seneviratne SL, Burns SO, Hurst JR. Corticosteroid-induced remission and mycophenolate maintenance therapy in granulomatous lymphocytic interstitial lung disease: long-term, longitudinal change in lung function in a single-centre cohort. ERJ Open Res. 2022 Oct 17;8(4):00024-2022. doi: 10.1183/23120541.00024-2022. PMID: 36267899; PMCID: PMC9574553.

[83] Tessarin G, Baronio M, Gazzurelli L, Rossi S, Chiarini M, Moratto D, Giliani SC, Bondioni MP, Badolato R, Lougaris V. Rituximab Monotherapy Is Effective as First-Line Treatment for Granulomatous Lymphocytic Interstitial Lung Disease (GLILD) in CVID Patients. J Clin Immunol. 2023 Nov;43(8):2091-2103. doi: 10.1007/s10875-023-01587-4. Epub 2023 Sep 27. PMID: 37755605; PMCID: PMC10661825.

[84] Ng J, Wright K, Alvarez M, Hunninghake GM, Wesemann DR. Rituximab Monotherapy for Common Variable Immune Deficiency-Associated Granulomatous-Lymphocytic Interstitial Lung Disease. Chest. 2019 May;155(5):e117-e121. doi: 10.1016/j.chest.2019.01.034. PMID: 31060706; PMCID: PMC6689079.

[85] Lamers OAC, Smits BM, Leavis HL, de Bree GJ, Cunningham-Rundles C, Dalm VASH, Ho HE, Hurst JR, IJspeert H, Prevaes SMPJ, Robinson A, van Stigt AC, Terheggen-Lagro S, van de Ven AAJM, Warnatz K, van de Wijgert JHHM, van Montfrans

J. Treatment Strategies for GLILD in Common Variable Immunodeficiency: A Systematic Review. Front Immunol. 2021 Apr 15;12:606099. doi: 10.3389/fimmu.2021.606099. PMID: 33936030; PMCID: PMC8086379.

[86] Pecoraro A, Crescenzi L, Galdiero MR, Marone G, Rivellese F, Rossi FW, de Paulis A, Genovese A, Spadaro G. Immunosuppressive therapy with rituximab in common variable immunodeficiency. Clin Mol Allergy. 2019 May 6;17:9. doi: 10.1186/s12948-019-0113-3. PMID: 31080365; PMCID: PMC6501382.

[87] Chase NM, Verbsky JW, Hintermeyer MK, Waukau JK, Tomita-Mitchell A, Casper JT, Singh S, Shahir KS, Tisol WB, Nugent ML, Rao RN, Mackinnon AC, Goodman LR, Simpson PM, Routes JM. Use of combination chemotherapy for treatment of granulomatous and lymphocytic interstitial lung disease (GLILD) in patients with common variable immunodeficiency (CVID). J Clin Immunol. 2013 Jan;33(1):30-9. doi: 10.1007/s10875-012-9755-3. Epub 2012 Aug 29. PMID: 22930256; PMCID: PMC3557581.

[88] Martelius T, Seppänen MRJ. Glimpses into treating GLILD. J Allergy Clin Immunol. 2023 Aug;152(2):365-367. doi: 10.1016/j.jaci.2023.06.010. Epub 2023 Jun 21. PMID: 37352977.

[89] Franxman TJ, Howe LE, Baker JR. Infliximab for Treatment of Granulomatous Disease in Patients with Common Variable Immunodeficiency. J Clin Immunol. 2014;34(7):820-827. doi:10.1007/s10875-014-0079-3.

[90] Hill AT, Thompson RA, Wallwork J, Stableforth DE. Heart lung transplantation in a patient with end stage lung disease due to common variable immunodeficiency. Thorax. 1998;53(7):622-623. doi:10.1136/thx.53.7.622

[91] Whelan TPM. Lung Transplantation for Interstitial Lung Disease. Clin Chest Med. 2012;33(1):179-189. doi:10.1016/j.ccm.2011.12.003

[92] Stanojevic S, Kaminsky DA, Miller MR, Thompson B, Aliverti A, Barjaktarevic I, Cooper BG, Culver B, Derom E, Hall GL, Hallstrand TS, Leuppi JD, MacIntyre N, McCormack M, Rosenfeld M, Swenson ER. ERS/ATS technical standard on interpretive strategies for routine lung function tests. Eur Respir J. 2022 Jul 13;60(1):2101499. doi: 10.1183/13993003.01499-2021. PMID: 34949706.

[93] Eaton WW, Rose NR, Kalaydjian A, Pedersen MG, Mortensen PB. Epidemiology of autoimmune diseases in Denmark. J Autoimmun. 2007 Aug;29(1):1-9. doi: 10.1016/j.jaut.2007.05.002. Epub 2007 Jun 19. PMID: 17582741; PMCID: PMC2717015.

[94] Boelaert K, Newby PR, Simmonds MJ, Holder RL, Carr-Smith JD, Heward JM, Manji N, Allahabadia A, Armitage M, Chatterjee KV, Lazarus JH, Pearce SH, Vaidya B, Gough SC, Franklyn JA. Prevalence and relative risk of other autoimmune diseases in subjects with autoimmune thyroid disease. Am J Med. 2010 Feb;123(2):183.e1-9. doi: 10.1016/j.amjmed.2009.06.030. PMID: 20103030.

[95] Thorarinsdottir K, Camponeschi A, Gjertsson I, Mårtensson IL. CD21-/low B cells: A Snapshot of a Unique B Cell Subset in Health and Disease. Scand J Immunol. 2015;82(3):254-261. doi:10.1111/sji.12339

[96] Hwangpo T, Wang Z, Ghably J, Bhatt SP, Cui X, Schroeder HW Jr. Use of FEF25-75% to Guide IgG Dosing to Protect Pulmonary Function in CVID. J Clin Immunol. 2020;40(2):310-320. doi:10.1007/s10875-019-00730-4

[97] Kollert F, Venhoff N, Goldacker S, Wehr C, Lützen N, Voll RE, Prasse A, Warnatz K. Bronchoalveolar lavage cytology resembles sarcoidosis in a subgroup of granulomatous CVID. Eur Respir J. 2014 Mar;43(3):922-4. doi: 10.1183/09031936.00025513. PMID: 24585875.