INTERSTITIAL LUNG DISEASE IN PRIMARY IMMUNODEFICIENCIES

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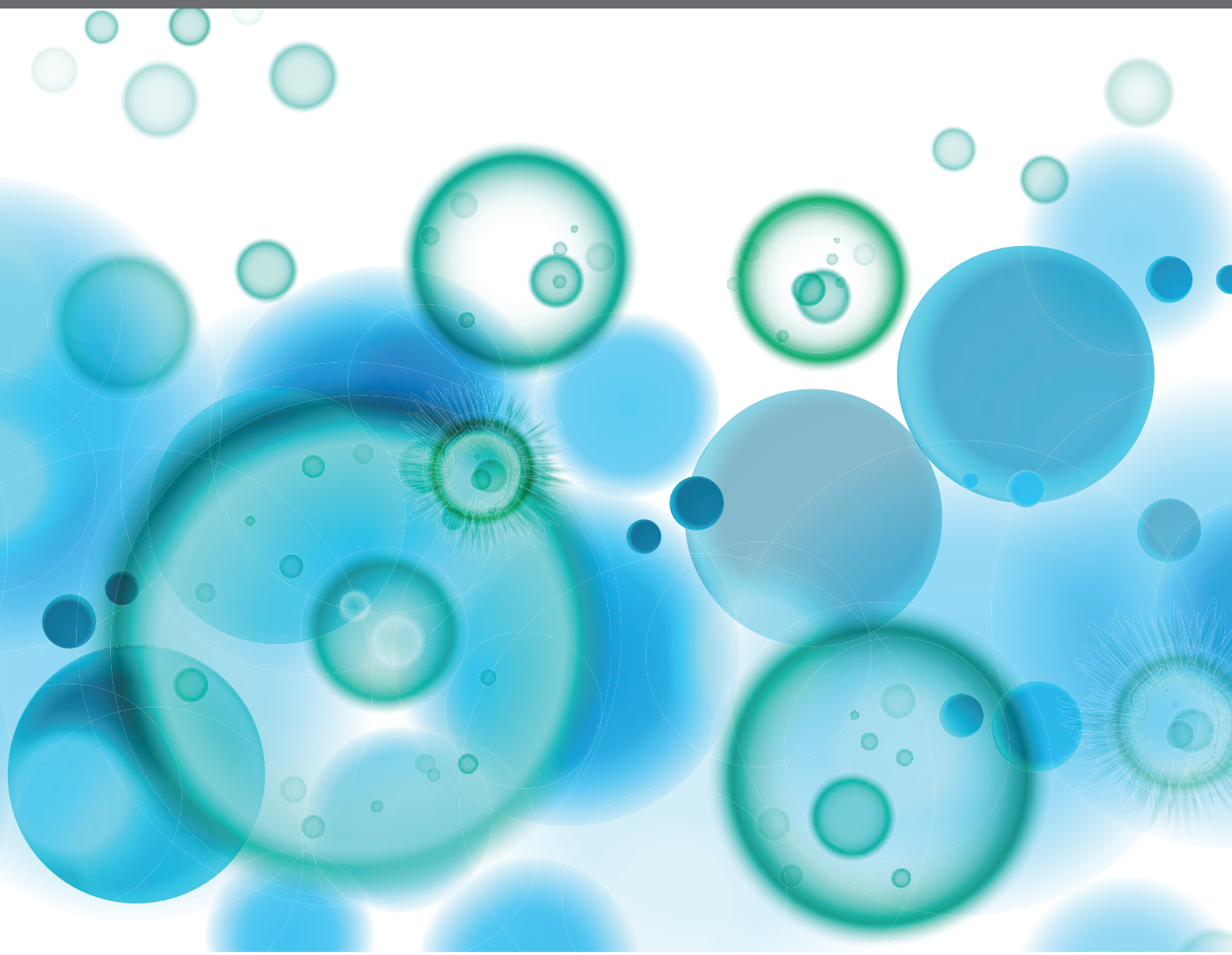

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INTERSTITIAL LUNG DISEASE IN PRIMARY IMMUNODEFICIENCIES

Topic Editors:
Børre Fevang, Oslo University Hospital, Norway
John R. Hurst, University College London, United Kingdom
Klaus Warnatz, University of Freiburg, Germany

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Table of Contents

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EDITORIAL
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Editorial: Interstitial Lung Disease in Primary Immunodeficiencies

Børre Fevang[1,2,3]*, *Klaus Warnatz*[4,5] *and John R. Hurst*[6]

[1] Section of Clinical Immunology and Infectious Diseases, Oslo University Hospital, Oslo, Norway, [2] Research Institute of Internal Medicine, Oslo University Hospital, Oslo, Norway, [3] Centre for Rare Diseases, Oslo University Hospital, Oslo, Norway, [4] Department of Rheumatology and Clinical Immunology, Medical Center- University of Freiburg, Faculty of Medicine, University of Freiburg, Freiburg, Germany, [5] Center for Chronic Immunodeficiency, Medical Center- University of Freiburg, Faculty of Medicine, University of Freiburg, Freiburg, Germany, [6] UCL Respiratory, University College London, London, United Kingdom

Keywords: interstitial lung disease (ILD), primary immunodeficencies (PID), common variable immune deficiency (CVID), granulomatous lymphocytic interstitial lung disease (GLILD), APECED, Autoimmune polyendocrinopathy candidiasis ectodermal dystrophy

Editorial on the Research Topic

Interstitial Lung Disease in Primary Immunodeficiencies

Primary immunodeficiencies (PID) are a heterogeneous group of disorders characterized not only by increased risk of infections but also by immune dysregulation affecting a number of organs, including the lungs (1). Interstitial lung disease (ILD) in PID can therefore be considered as the pulmonary manifestation of a systemic immune dysregulation, and can be a serious threat to the health of afflicted patients (2, 3). The condition has been called granulomatous-lymphocytic interstitial lung disease (GLILD) although this term is unsatisfactory. This collection includes a broad range of articles addressing clinical, immunological and radiological features of ILD in PID.

Overall, the articles underscore the need for standardization of clinical practice and research. This is clearly shown by Van De Ven et al. presenting the findings of an international survey among pulmonologists and immunologists characterizing clinical practice and main challenges faced in care and research on GLILD. Out of 161 respondents from 47 countries only 19% had access to a standardized protocol for diagnosis and treatment. Overall, there was a wide variety in the interventions taken and the authors strongly argue for more standardized clinical studies on GLILD. Interestingly, while 71% of respondents would not routinely undertake biopsy for the diagnosis of GLILD, 46 out of 103 respondents stated that alternative diagnoses had been found on biopsies (not necessarily taken on routine), including lymphoma.

The issue of histopathological diagnosis is further explored in the review article by Dhalla et al. They argue for the need of standardization of histopathological findings to bring the understanding of the basic pathophysiology forward. There is currently considerable variation in histopathological findings between studies and we do not know if this represents biopsy-related factors or that ILD in CVID represents a spectrum of diseases, separate diseases or a shared endpoint for several diseases. An alternative strategy to understand the underlying pathophysiology can be to study bronchoalveolar lavage fluid (BAL-F). In their original article, Friedman et al., analyze findings of BAL-F from patients with common variable immunodeficiency disorders (CVID), sarcoidosis and healthy controls. They find a mixed expansion of lymphocytes in BAL-F from CVID-patients dominated by Th1-cells and CD21low B-cell while levels of regulatory T cells were low. There were

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Edited and reviewed by:
Isabelle Meyts,
KU Leuven, Belgium

***Correspondence:**
Børre Fevang
borre.fevang@medisin.uio.no

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also low levels of Th17-cells even if IL-17 was upregulated together with the B-cell activating factor APRIL. Mechanisms of B-cell activation, maturation and survival in the lung of affected patients are further discussed in the review article by Matson et al. The B-cell activation factor (BAFF) signaling through BAFF-R, TACI and BCMA has been shown to be associated with both presence and recurrence of ILD in CVID. The authors recommend further studies on the IFN-g/STAT1/BAFF axis.

While most articles in this topic focus on CVID, Ferré and Lionakis in their review article highlights ILD as a relevant complication of the immunodysregulatory disease Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED). APECED can be caused by various mostly biallelic mutations in the *AIRE*-gene. Clinically and radiologically, ILD in APECED shares features with other forms of ILD, and interestingly pulmonary biopsies show a pattern of T- and B-cell infiltrates. APECED patients produce a variety of autoantibodies, including anti-BPIFB1 and anti-KCNRG that are associated with pulmonary disease. While treatment with mycophenolate and rituximab have clear clinical and radiologic effects, levels of the two autoantibodies are not affected, suggesting B-cells contribute to ILD through varied pathways, including priming of T-cells.

The issue of ILD is often raised through radiological examination and several articles look into this. Meerburg et al. examined CT scans from 138 GLILD-patients included in the STILPAD-study comparing the Baumann and Hartmann scoring methods. Both methods systematically score radiologic features of GLILD and detected the presence of features of GLILD in >95% of patients with high reproducibility, especially for the Hartmann method. The Hartmann method evaluates abnormalities in more detail than the Baumann method but is too laborious (time needed per CT scan, 30 *vs* 15 minutes, respectively) for daily clinical practice. Fraz et al., present a systematic evaluation of findings of CT and PET/CT in a cohort of 32 CVID patients with radiologic features of GLILD and relate them to clinically progressive and stable disease. Patients with progressive disease had significantly higher overall score of pathologic features on CT and higher SUV uptake on PET/CT compared to patients with clinically stable disease. Treatment with rituximab was associated with significant improvement in pathologic features while the effect on lung function measured by forced vital capacity and CO diffusion were variable.

Using data from 7 Italian PID centers Cinetto et al. present radiological, clinical and immunological findings in a cohort of 75 CVID patients with radiologic features of ILD and compare them to 125 CVID controls. The patients with radiologic ILD-findings were further divided into patients with GLILD based on histology of lung or other tissue, or undetermined (u)ILD based on a clinical-radiological diagnosis without biopsy. Patients with GLILD and to a lesser extent uILD were characterized by splenomegaly, autoimmune cytopenia, low DLCO and high frequency of CD21low B-cells. Pooling these features together the authors made a predictive model for GLILD with a ROC curve of 0.98, possibly limiting the need for diagnostic biopsies for GLILD. Lopes et al. present clinical, immunologic and radiologic data on 46 patients with biopsy-proven ILD. They find a rate of granulomas above 50% in pulmonary tissue but also high frequency of lymphoid interstitial pneumonia. Nine patients died during the observation period with a median age

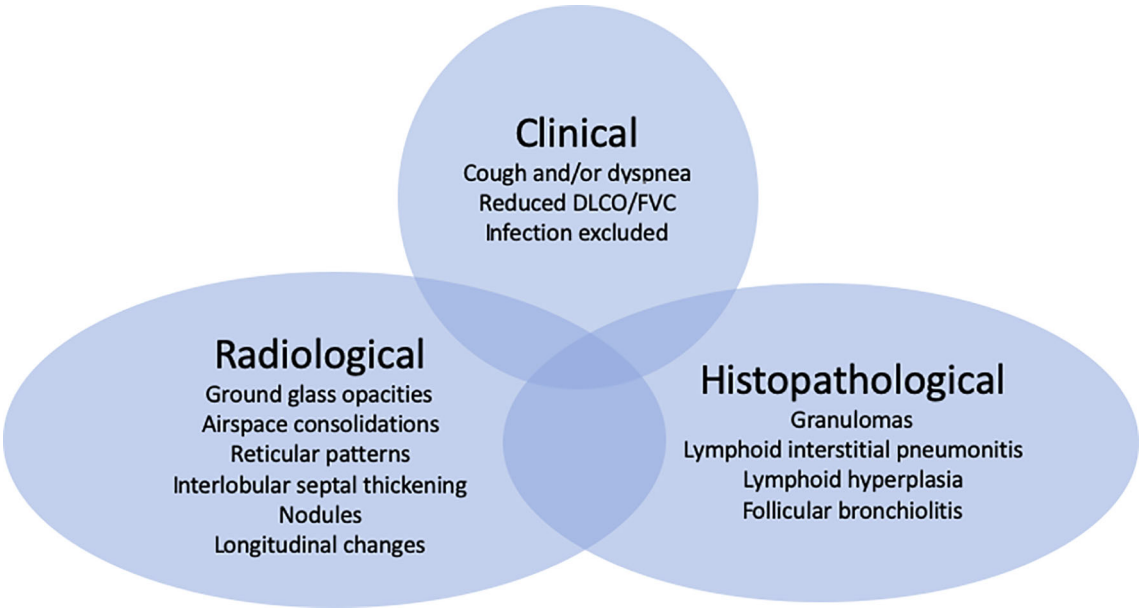

FIGURE 1 | (GL) ILD in CVID is characterized by clinical, radiological and histopathological features with patients presenting all or some of these manifestations. Which criteria to include for the diagnosis demands an imminent debate (4).

of death of 49 years underscoring the serious nature of this complication in CVID.

There is therefore a clear need for better treatment of ILD in PID, and in their systematic review article, Lamers et al., aim to summarize and synthesize literature on efficacy of treatments for GLILD. They find 41 papers describing case series or uncontrolled studies reporting on 255 patients. The heterogeneity characterizing publications on GLILD makes comparing studies difficult but there was a trend towards more relapses in patients treated with glucocorticoids only. van Stigt et al. approach treatment of GLILD through looking at treatment of granulomatous disease of CVID in general in their review article. Extra-pulmonary disease has been reported in lymph nodes, spleen, gastrointestinal tract, bone marrow, liver and skin among others. Reports of 95 CVID-patients treated for granulomatous disease were identified in literature (45 patients with extra-pulmonary disease only, 51 patients with pulmonary granulomatous disease) receiving a total of 117 different treatment courses. While steroid monotherapy is used for all granulomatous disease, it is reported more frequently for extrapulmonary disease (21/53 *vs* 15/64 courses, extrapulmonary and pulmonary disease, respectively) and with remission in 85.7% of cases. Anti-TNF therapy was also more frequently reported in extrapulmonary disease, while rituximab and azathioprine were administered almost solely in pulmonary disease.

This Research Topic will hopefully inspire centers around the world to collaboratively tackle this field. An important first milestone will be to agree on which criteria to base the diagnosis of ILD in PIDs (**Figure 1**).

AUTHOR CONTRIBUTIONS

BF, KW, and JH all contributed to the planning and writing of this manuscript. BF wrote the first draft, which was then revised by KW and JH. All authors contributed to the article and approved the submitted version.

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ORIGINAL RESEARCH
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Analysis of Granulomatous Lymphocytic Interstitial Lung Disease Using Two Scoring Systems for Computed Tomography Scans—A Retrospective Cohort Study

Jennifer J. Meerburg[1,2], Ieneke J. C. Hartmann[3], Sigune Goldacker[4], Ulrich Baumann[5], Annette Uhlmann[6], Eleni-Rosalina Andrinopoulou[7], Mariette P. C. Kemner v/d Corput[1,2], Klaus Warnatz[4,8†] and Harm A. W. M. Tiddens[1,2†] on behalf of the STILPAD study group*

[1] Department of Paediatric Pulmonology and Allergology, Sophia Children's Hospital—Erasmus Medical Center, Rotterdam, Netherlands, [2] Department of Radiology and Nuclear Medicine, Erasmus Medical Center, Rotterdam, Netherlands, [3] Department of Radiology, Maasstad Hospital, Rotterdam, Netherlands, [4] Department of Rheumatology and Clinical Immunology, Faculty of Medicine, University of Freiburg, Medical Center—University of Freiburg, Freiburg, Germany, [5] Department of Paediatric Pulmonology, Allergy and Neonatology, Hannover Medical School, Hannover, Germany, [6] Institute for Immunodeficiency, Center for Chronic Immunodeficiency (CCI), Medical Center - University of Freiburg, Faculty of Medicine, University of Freiburg, Freiburg, Germany, [7] Department of Biostatistics, Erasmus Medical Center, Rotterdam, Netherlands, [8] Center for Chronic Immunodeficiency (CCI), Faculty of Medicine, University of Freiburg, Medical Center—University of Freiburg, Freiburg, Germany

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Edited by:
Antonio Condino-Neto, University of São Paulo, Brazil

Reviewed by:
David Andrew Fulcher, Australian National University, Australia
Anders Fasth, University of Gothenburg, Sweden

***Correspondence:**
Harm A. W. M. Tiddens
h.tiddens@erasmusmc.nl

†These authors share joint last authorship

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Background: Granulomatous lymphocytic interstitial lung disease (GLILD) is present in about 20% of patients with common variable immunodeficiency disorders (CVID). GLILD is characterized by nodules, reticulation, and ground-glass opacities on CT scans. To date, large cohort studies that include sensitive CT outcome measures are lacking, and severity of structural lung disease remains unknown. The aim of this study was to introduce and compare two scoring methods to phenotype CT scans of GLILD patients.

Methods: Patients were enrolled in the "Study of Interstitial Lung Disease in Primary Antibody Deficiency" (STILPAD) international cohort. Inclusion criteria were diagnosis of both CVID and GLILD, as defined by the treating immunologist and radiologist. Retrospectively collected CT scans were scored systematically with the Baumann and Hartmann methods.

Results: In total, 356 CT scans from 138 patients were included. Cross-sectionally, 95% of patients met a radiological definition of GLILD using both methods. Bronchiectasis was present in 82% of patients. Inter-observer reproducibility (intraclass correlation coefficients) of GLILD and airway disease were 0.84 and 0.69 for the Hartmann method and 0.74 and 0.42 for the Baumann method.

Conclusions: In both the Hartmann and Baumann scoring method, the composite score GLILD was reproducible and therefore might be a valuable outcome measure in future studies. Overall, the reproducibility of the Hartmann method appears to be slightly better

than that of the Baumann method. With a systematic analysis, we showed that GLILD patients suffer from extensive lung disease, including airway disease. Further validation of these scoring methods should be performed in a prospective cohort study involving routine collection of standardized CT scans.

Clinical Trial Registration: https://www.drks.de, identifier DRKS00000799.

Keywords: computed tomography, interstitial lung disease, common variable immune deficiency (CVID), cohort study (or longitudinal study), airway disease, granuloma, scoring systems

INTRODUCTION

Common variable immunodeficiency disorders (CVID) are a heterogeneous group of primary antibody deficiency syndromes (1). Clinical diagnosis is based on a decreased level of IgG, IgA, and/or IgM, an impaired immune response to vaccines, and the absence of defined causes for hypogammaglobinaemia (2). CVID result in a broad spectrum of clinical presentations (3). In the early stages of disease, patients often present with recurrent upper and lower respiratory tract infections. Although the use of immunoglobulin replacement therapy can significantly reduce the risk of lower respiratory tract infection in these patients (4), a substantial proportion of patients develop progressive airway disease (5, 6).

In addition, 30%–50% of CVID patients develop non-infectious autoimmune disease, organ inflammation or malignancies. Since adequate immunoglobulin replacement therapy has been introduced, these comorbidities have a larger impact on patient prognosis than the recurrent infections (3, 7). Granulomatous lymphocytic interstitial lung disease (GLILD) belongs to these comorbidities and affects 8%–20% of CVID patients (8, 9). GLILD patients show signs of lymphoproliferative pulmonary disease, including lymphocytic interstitial pneumoniae, follicular bronchiolitis, or lymphoid hyperplasia in combination with granulomas. The diagnosis is made by performing both radiological and histopathological examinations of the lungs (6, 9). Although the pathogenesis of GLILD is not well understood, autoimmune and inflammatory dysregulation and their association with other autoimmune disorders are thought to play a role (5). It was shown that CVID patients with GLILD (n = 13) have a markedly reduced survival rate of 50% compared to patients without GLILD (n = 56) and this finding led to a heightened clinical interest in the GLILD patient group (9). Importantly, this interstitial lung disease can lead to clinical complaints such as reduced exercise tolerance and dyspnoea. Furthermore, GLILD patients have a more complex clinical course, as they tend to have a higher frequency of B-cell lymphoma and autoimmune diseases compared to non-GLILD patients (9, 10). Currently, the gold standard to assess GLILD-related structural lung changes is chest computed tomography (CT). Frequently observed lung abnormalities in GLILD include: ground-glass opacities (GGO), diffuse nodules, lymphadenopathy, diffuse patchy consolidations, and reticulation (9, 11, 12). This is distinct from signs of airway disease, like bronchiectasis, airway wall thickening and trapped air (11, 13–16). Two typical CT images of GLILD patients are shown in **Figure 1**.

Most studies on GLILD-related CT structural lung abnormalities involve retrospectively extracted data from radiologic reports (9, 12, 17–19). However, these reports are generally not well standardized nor quantitative, making it difficult to compare findings.

A more systematic and reproducible approach to quantify these abnormalities is to use standardized CT scoring methods. Outcome measures derived from scoring methods can be used both for research purposes and in clinical follow-up (20). Furthermore, they can be used to phenotype patients for personalized clinical care. Few studies have employed scoring methods to systematically assess chest CT scans of GLILD

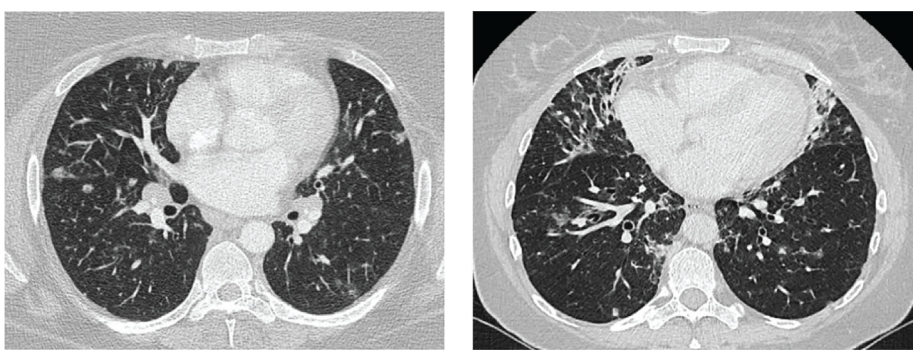

FIGURE 1 | Features of granulomatous lymphocytic interstitial lung disease (GLILD). Images of two study patients. Left: diffuse nodules and lymphadenopathy. Right: combination of diffuse nodules, reticulation and ground-glass opacities. Apart from GLILD features, also signs of airway disease.

patients. Van de Ven et al. used a scoring system for paediatric CVID or CVID-like patients (n = 54), which was subsequently applied to adults with CVID (n = 47) (15, 21). Similarly, Gregersen et al. used a simplified scoring method to assess CVID in adults (n = 65) (22). Chase et al. evaluated the efficacy of chemotherapy in seven GLILD patients by assessing CT scans performed before and after treatment (23). A major limitation of these studies is that only a small number of patients with GLILD were included. This warrants the need for larger cohort studies to better understand the radiologic characteristics of GLILD and to optimise methods to quantify disease severity in these patients (6, 9, 24).

From 2012 to 2014, a large international observational study, The STudy of Interstitial Lung Disease in Primary Antibody Deficiency (STILPAD), was initiated by the Centre of Chronic Immunodeficiency at the University Medical Centre Freiburg in Freiberg, Germany. The purpose of STILPAD was to describe the natural course and different treatment responses of GLILD. Fourteen medical centers across three countries retrospectively collected clinical data of 146 GLILD patients, from which all available chest CT scans were analyzed to phenotype pulmonary abnormalities in these patients. The aim of this present study was to assess the radiologic features on retrospectively collected chest CT scans of the STILPAD subjects using and comparing two independent scoring methods developed for CVID patients.

METHODS AND MATERIALS

Study Population

Patients with the clinical diagnosis of GLILD enrolled in STILPAD between 2012 and 2014 were included in this study. Inclusion criteria were as follows: 1) CVID defined by criteria approved by the European Society for Immunodeficiencies and the Pan-American Group for Immunodeficiency (2), 2) age of 18 years and above, and 3) a radiological diagnosis of interstitial lung disease or granuloma on chest CT scan, characterized by the presence of nodules, reticulation, or GGO. This evaluation was performed by the radiologist at each participating medical center.

Given the unresolved discussion whether a histological proof of GLILD is required, a histopathological diagnosis of GLILD was made only in few patients based on the policy of each center, and this was not an inclusion criterion.

Collection of CT Scans

All available digital CT scans of the STILPAD cohort were collected retrospectively between December 2013 and April 2015. Exclusion criteria for image analysis were as follows: incomplete display of the lung, substantial motion artefacts, pneumothorax, or the absence of a reconstruction series required for lung image analysis. To evaluate the presence and severity of pulmonary abnormalities in GLILD patients, the most recent CT scan of each patient was analyzed. For the assessment of change in disease over time, patients with at least two CT scans were included.

CT Scan Characteristics

Information on CT parameters, including slice thickness, lung volume during acquisition, volumetric or sequential acquisition, and the reconstruction kernels were noted for each scan.

CT Scan Analysis

CT scans were scored using two methods developed for scoring CVID CT scans: the Baumann method and the Hartmann method. Key features of these methods are outlined in **Table 1**. Both scoring methods evaluate not only CT changes associated with interstitial lung disease but also airway disease as outlined below.

Baumann Scoring Method

The Baumann scoring method, shown in **Supplemental Digital Content 1**, was developed by an international interdisciplinary group known as the Chest CT Antibody Deficiency Group. One of its objectives is to standardise the reporting of chest CT findings of patients with antibody deficiencies in a reproducible and clinically applicable manner. The group recently published a report on the distribution of bronchial pathologies in CVID patients in a large international cohort (25). The Baumann method evaluates the presence of 13 different abnormalities without assessing their distribution within specific

TABLE 1 | Differences between the Baumann and Hartmann scoring methods for common variable immunodeficiency disorders.

	Baumann	Hartmann
Abnormalities scored per	Whole lung	Lobe
Number of values	22	157
Time needed per CT (minutes)	15	30
Origin	Newly designed for CVID as a scoring system for clinical use	Based on the cystic fibrosis-CT scoring method, and designed as a sensitive scoring system for CVID patients for research purposes
Emphysema	Scored together with bullae	Scored as separate entity
Reticulation	The presence and subtype of reticulation (inflammatory, fibrotic, or mixed) are noted	Differentiation between reticulation with or without distortion
Lymphadenopathy	Size of the largest lymph node is measured in mm	Only the presence is scored defined by a short axis diameter ≥ 10 mm
Ground-glass opacities (GGO)	Both the presence and subtype of GGO (inflammatory or fibrotic) are noted	No subtypes of GGO are noted

This table presents the key differences between the Baumann and Hartmann scoring method for computed tomography (CT) scans of patients with common variable immunodeficiency disorders (CVID).

lobes of the lung. These include: bronchial wall thickening, bronchiectasis (excluding traction bronchiectasis), mucus plugging, atelectasis, nodules, reticulation ("lines"), consolidation, GGO, cysts, emphysema or bullae, linear scars and bands, trapped air, and lymphadenopathy. Briefly, the extent of each abnormality is evaluated by counting the number of affected lung lobes; the lingula being considered as a separate lobe. Furthermore, a score between 0 and 3 denotes the severity of bronchial wall thickening and bronchiectasis. Nodules are divided into three size-based categories and in cases of lymphadenopathy; the size of the largest lymph node is measured. This results in 22 scoring items per CT-scan.

Hartmann Scoring Method

The Hartmann scoring method, shown in **Supplemental Digital Content 2**, is derived from the validated cystic fibrosis - CT scoring method, with additional items describing abnormalities typical of immunodeficiency syndromes (26). The Hartmann method evaluates abnormalities in more detail than the Baumann method to detect more subtle changes over time. This method was designed for research purposes and is less suitable for clinical practice due to its extensiveness. In summary, the following abnormalities are assessed: bronchial wall thickening, bronchiectasis (excluding traction bronchiectasis), mucus plugging, atelectasis, nodules, reticulation, consolidation, GGO, bullae and cysts, emphysema, distortion, trapped air, and lymphadenopathy. Unlike the Baumann method, each lobe is scored separately, with the lingula being considered as a separate lobe. The extent and severity of specific abnormalities are scored on a scale of 0 to 3. A total of 26 items are scored per lobe, and lymphadenopathy is only scored once. This results in 157 scoring items per CT scan.

Component and Composite Scores

In both methods, individual component scores for bronchiectasis, bronchial wall thickening, mucus plugging, nodules, reticulation and GGO are expressed as a percentage of the maximum score.

Component scores of bronchiectasis and bronchial wall thickening were calculated by multiplying the extent of disease by a factor (multiplier), such that the higher the severity of disease, the higher the multiplier (27, 28). Bronchiectasis severity scores of 1.0, 1.5, 2.0, 2.5, and 3.0 had multipliers of 1.00, 1.25, 1.50, 1.75, and 2.00, respectively. Likewise, bronchial wall thickening scores of 1.0, 2.0, and 3.0 had respective multipliers of 1.00, 1.25, and 1.50.

Besides the component scores for single abnormalities, three composite scores were calculated and expressed as a percentage of the maximum score. The GLILD composite score comprised the combined score of GGO, nodules, and reticulation. The composite score for airway disease consisted of bronchial wall thickening, bronchiectasis and mucus plugging combined. In addition, the total disease composite score was derived from the sum of all scored abnormalities.

In case no signs of GLILD were found with both the Baumann and Hartmann scores, the CT scans were analyzed by a thoracic radiologist (P.C.).

Observers

The CT scans were scored by two extensively trained observers (a medical doctor and a final year medical student). Observers were trained and certified using standardized chest CT training modules that were developed by a chest radiologist (IH) and the LungAnalysis Core Laboratory. These modules consist of studying a defined list of literature (29), followed by PowerPoint presentations to train used definitions and reference images to be used for scoring. Finally, the observers had to score training batches of CT scans. Furthermore, each observer received one-to-one training sessions with the chest radiologist (IH). For logistical reasons, the scans were divided into two batches (n = 251 and n = 105), based on order of arrival. Each batch was scored by a single observer. To assess inter- and intra-observer reliability each observer re-scored a randomly selected and randomized batch of 25 and 30 CT scans, respectively.

Statistical Analysis

Patient demographics are reported as mean (standard deviation) and scoring outcome parameters are presented as the median (interquartile range, total ranges).

Agreement within and between observers was determined using the intraclass correlation coefficients (ICCs) of both scoring methods (two-way mixed-effects model, single measurements, studied relationship consistency) (30). ICC ranges are defined as follows: 0–0.39 poor, 0.40–0.59 fair, 0.60–0.74 good, and >0.75 excellent (31).

To investigate changes in disease over time, mixed-effects models (generalized estimating equations) were used for the following CT outcomes of both scoring methods: the component score bronchiectasis and component scores GLILD, and airway disease and total disease scores. Models were adjusted for multiple visits, with p-values <0.05 considered significant.

Square root-transformed Hartmann component scores of bronchiectasis were used, as the assumption of homoscedasticity (constant variance) was not satisfied in the original scale. Likelihood-ratio tests were used to assess whether a nonlinear assumption would better represent the evolution of disease over time.

Statistical analyses were performed using SPSS version 21.0 (SPSS Inc., Chicago, IL) and R version 3.3.1 (https://cran.r-project.org/).

Ethics Approval

Approval for this study was obtained from the local ethics committee of the University of Freiburg in Freiburg, Germany (IRB: 189/12), and the national ethical review boards of all participating centers. Written informed consent was obtained from all participants prior to inclusion in this study.

RESULTS

Study Population

For this CT analysis eight patients from the STILPAD cohort (n = 146) were excluded, because they had no digital CT scans

available (n = 7) or the available CT scans did not meet the inclusion criteria (n = 1). Hence, 138 patients were included in this retrospective CT study, of which 88 (64%) females. The mean age at time of inclusion was 45 (± 15) years, and mean age of diagnosis was 41 (± 15) years.

Collection of CT Scans

A total of 462 CT scans were collected. A flowchart of the CT scan selection process is shown in **Figure 2**. We excluded 105 CT scans as they failed to meet the inclusion criteria and one CT because it was unintentionally scored using only the Hartmann method. Ultimately, the final cohort compromised 356 CT scans from 138 patients.

For the longitudinal analysis, 299 scans were collected from 81 patients. **Figure 3** shows the number of CT scans that were analyzed per patient. Median interval (interquartile range, total range) between the CT scans was 12 months (5–24, 0–114).

CT Scan Characteristics

An overview of the scan characteristics is provided in **Digital Supplement Content 3**. In short: The majority of CT scans (n = 274, 77%) were volumetric. Slice thickness ranged between 0.6 and 8.0 mm, with 267 (75%) of scans having a slice thickness below 3.0 mm. Because only two expiratory CT scans could be collected, trapped air had to be excluded from the analysis.

CT Scan Analysis of the Most Recent CT

Presence of Abnormalities

Figures 4A, B display the prevalence of component and composite scores of GLILD and airway disease on the most recent CT scan using the Baumann and Hartmann scoring methods. Bronchiectasis was the most common abnormality, with a prevalence of 113 (82%) in all patients for both scoring methods. Other common findings include: bronchial wall thickening, GGO, reticulation and nodules. Signs of GLILD, as calculated by combining the scores of GGO, nodules and/or reticulation, were found on the most recent CT in 131 (95%) of patients for both methods. **Figure 5** demonstrates the relationships between GLILD features. In 56% and 60% of these

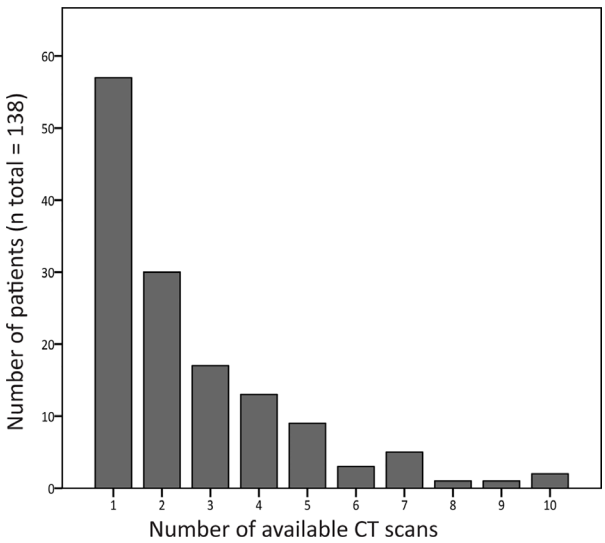

FIGURE 3 | Number of computed tomography (CT) scans available per patient. The number of CT scans that was analyzed per patient is shown in this graph. Of 81 patients, two or more CT scans were collected, and these scans were used for follow-up analysis.

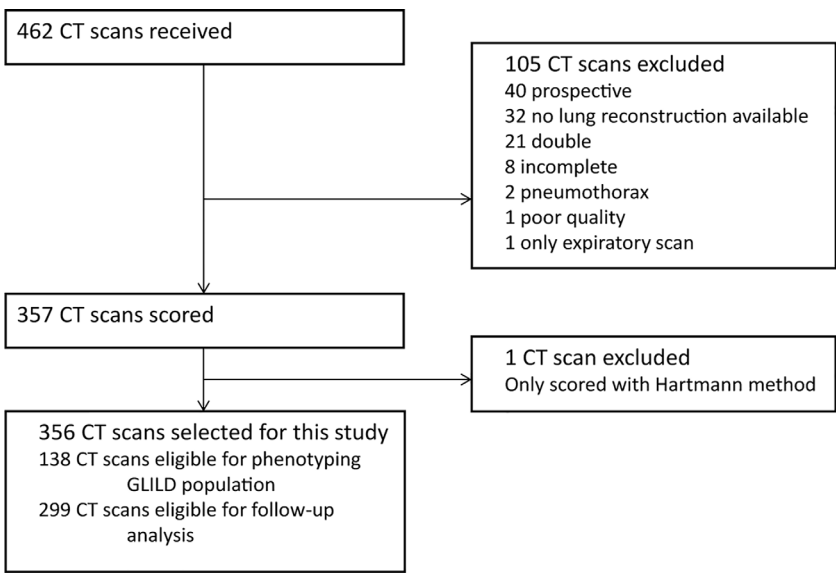

FIGURE 2 | Flowchart CT selection. Flowchart of the in- and exclusion of CT scans. GLILD, granulomatous lymphocytic interstitial lung disease. For phenotyping the GLILD population 138 most recent CT scans were used. A total of 356 CT scans from 138 STILPAD subjects were analyzed and selected for this study. The most recent scan of each patient was used to phenotype the GLILD population. For follow-up analysis, 299 CT scans from 81 patients were analyzed.

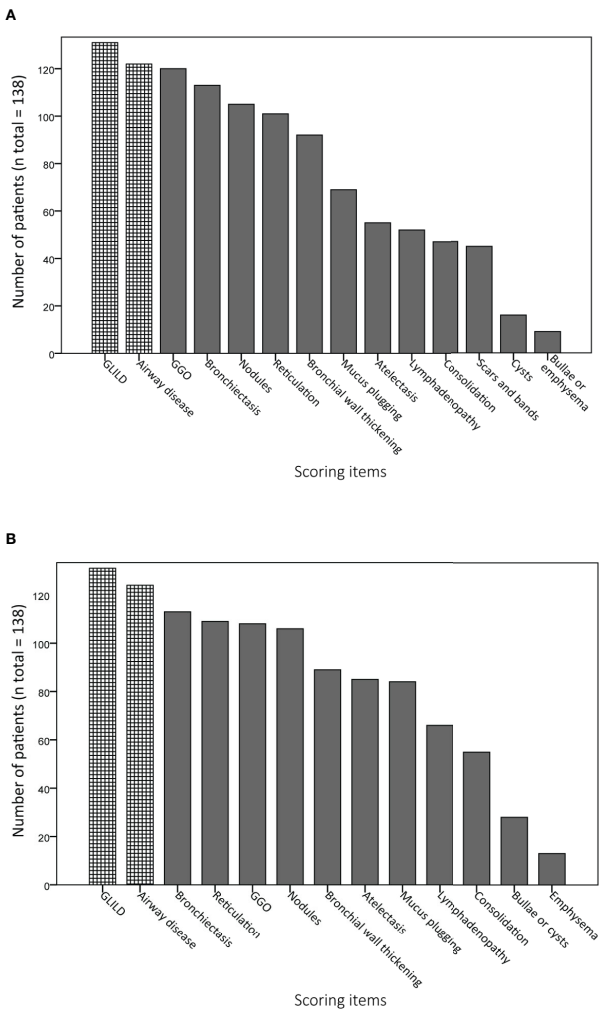

FIGURE 4 | Prevalence of abnormalities on computed tomography (CT) scan. Component and composite scores are sorted based on the number of patients that have a positive score. Granulomatous lymphocytic interstitial lung disease (GLILD) and airway disease are composite scores; GLILD is a combination of component scores for ground-glass opacities (GGO), nodules and reticulation, airway disease is the sum of bronchial wall thickening, bronchiectasis and mucus plugging component scores. **(A)** Scoring items Baumann method. **(B)** Scoring items Hartmann method.

patients, all features of GLILD were detected with the Baumann and Hartmann method respectively. Signs of GLILD were not detected on the most recent CT scan of five (4%) STILPAD patients in any of the two scoring methods. Of these patients, one patient (1%) had positive GLILD scores on previous scans. The CT scans of the four patients without positive GLILD composite scores on any of their CT scans were re-evaluated by a thoracic radiologist, and signs of GLILD were detected in two of the four patients. Airway disease, defined as bronchiectasis and/or bronchial wall thickening and/or mucus plugging, was present in 122 (88%) (Baumann) and 124 (90%) (Hartmann) of patients. Enlarged lymph nodes were found in 52 (38%) (Baumann) and 70 (51%) (Hartmann) of patients.

Severity of Abnormalities

The maximal severity scores for bronchiectasis, bronchial wall thickening and nodules are presented in **Table 2**. Mild bronchiectasis and mild bronchial wall thickening were most frequently observed. In addition, the maximum severity score for bronchial wall thickening was never reached. If nodules were present, the diameter of the largest nodule exceeded the size of 5 mm in 89 (85%) (Baumann) and 87 (83%) (Hartmann) of patients.

Component and Composite Scores

Component scores (bronchiectasis, bronchial wall thickening, mucus plugging, nodules, reticulation, and GGO) and composite scores for airway disease, GLILD, and total disease (comprising all parameters) are shown in **Table 3**. The range between minimum and maximum scores using the Baumann method was wide, particularly for the component scores of bronchiectasis, nodules, GGO, reticulation, and the composite score GLILD which ranged between 0% and 100%. Differences in scores assessed with the Hartmann method were in a lower range compare to the Baumann method, and only the component score for nodules reached a maximum of 100%.

Longitudinal Analysis

Longitudinal analysis of all follow up scans (n = 299) using generalized estimating equation models showed that the squared root-transformed Hartmann bronchiectasis component score increased significantly over time (p = 0.0097). We found no statistically significant longitudinal change in the Baumann bronchiectasis component score and the Baumann and Hartmann composite scores for GLILD, airway disease, and total disease. Prediction plots of bronchiectasis component scores are presented in **Figure 6**. Complete statistical results of the analysis and prediction plots are displayed in **Supplemental Digital Content 4**.

Inter- and Intra-Observer Agreement

ICCs of the most common abnormalities are presented in **Table 4**. Both inter- as intra-observer agreement for the Hartmann method was for most items slightly higher than for the Baumann method. Between observers, the Hartmann component scores of reticulation and GGO only had poor inter-observer agreement, while within observers, the agreement for these items varied from poor to excellent. Of the component scores, nodules showed the highest agreement, while bronchial wall thickening and mucus plugging showed only poor to fair agreement. Subtypes of GGO (inflammatory or fibrotic) and reticulation (inflammatory, fibrotic, or mixed), which are exclusive to the Baumann method, showed a poor inter-observer agreement.

DISCUSSION

In this retrospective study, chest CT features of CVID patients with a radiological diagnosis of GLILD were described. A total of 356 CT scans of 138 patients were included and scored using

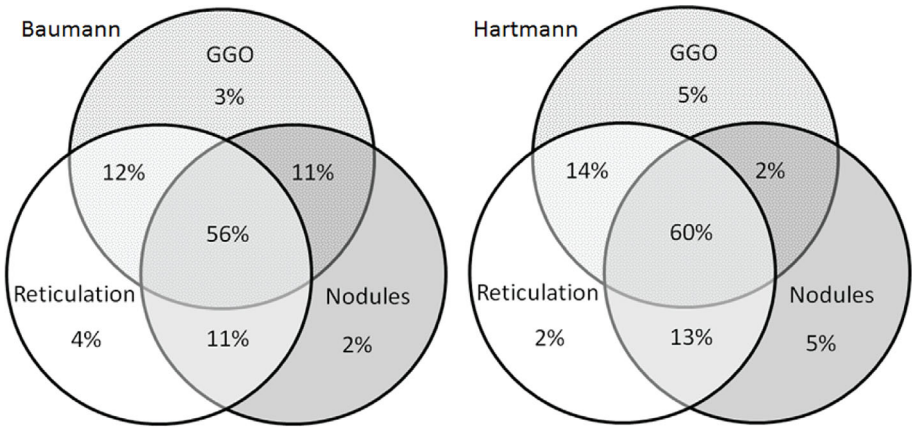

FIGURE 5 | Venn diagrams of features of granulomatous lymphocytic interstitial lung disease (GLILD). Venn diagrams showing the presence of the in the patients method with signs of GLILD on their most recent chest CT scan for both the Baumann (left) and Hartmann (right) (n total = 131). In 56% (Baumann) and 60% (Hartmann) of the 131 patients, all features of GLILD were detected. GGO, ground-glass opacities.

TABLE 2 | Severity of component scores, bronchiectasis, bronchial wall thickening, and nodules.

Severity of abnormalities	Baumann n (%)	Hartmann n (%)
Bronchiectasis (total)	113 (100)	113 (100)
Highest score of CT scan		
Airway >1–<2× vessel	93 (82)	73 (65)
Airway >2–<3× vessel	14 (12)	26 (23)
Airway > 3× vessel	6 (5)	14 (12)
Bronchial wall thickening (total)	92 (100)	89 (100)
Highest score of CT scan		
BW > 0.33–<0.5× vessel	85 (92)	75 (84)
BW >0.5–<1× vessel	7 (8)	14 (16)
BW > 1× vessel	0 (0)	0 (0)
Nodules (total)	105 (100)	106 (100)
Highest score of CT scan		
Largest nodule < 5 mm	16 (15)	19 (18)
Largest nodule >5–<10 mm	46 (44)	43 (41)
Largest nodule >10 mm	43 (41)	44 (42)

Maximal severity scores for the component scores bronchiectasis, bronchial wall thickening and nodules are presented for both methods. Numbers and percentages represent their distribution within the group on the most recent CT scan of patients (n = 138). CT, computed tomography; BW, bronchial wall.

two dedicated CVID scoring systems. A limitation of our study is that histopathological proof of GLILD was rarely available. However it seems that GLILD is not often misdiagnosed in clinical practice: Maglione et al. showed that in 15 of 61 patients in which biopsies were available, diagnosis did not change (16); and Mannina et al. demonstrated that there was no detectable difference between the patients biopsied and not biopsied in regard to the CT morphology or prognosis of the lung function (32). Furthermore, CT patterns compatible with the diagnosis of GLILD were confirmed by the evaluation of the independent readers in this study for all except four participants. Therefore, we consider the effect of lacking biopsy proven GLILD in regard to the goal of this study as minor.

Phenotyping GLILD Patients

The current pathogenic concept of GLILD comprises mixed T- and B-lymphocytic infiltration of the interstitium of the lungs, partly forming tertiary lymphoid structures next to granulomatous inflammation, follicular bronchiolitis, and reactive lymphoid hyperplasia (6, 33). Typical features of GLILD on CT are patchy GGO, both sharp and unsharp nodules, and reticular lesions varying from fine-lined to course (34). Of the full cohort of 138 included patients, these features were present on their most recent CT scan in 95% of patients, and when also older CT scans were included this was 97% of patients. The two patients, without detectable features of GLILD even after re-evaluation by a thoracic radiologist (P. Ciet), were likely to be misdiagnosed by the radiologists of the participating centers. Overall, this is quite a good result, since reported inter-observer agreement between thoracic radiologists for the diagnosis of general interstitial pneumonia, which has similarities with GLILD, was only 0.52. That of non-thoracic radiologists was even less, namely, 0.48 (35). In the patients with signs of GLILD on their most recent CT, only a small majority exhibited all key features of GLILD. In general, substantial heterogeneity of radiological features was observed in these patients. Enlarged lymph nodes were detected in only 38% of the patients for the Baumann score and in 51% for the Hartmann score. This low prevalence might be explained by the fact that intravenous contrast for better evaluation of lymph nodes was used in only half of the patients. There is no consensus whether contrast medium should be administered in these patients (36). The lower percentage of CTs with lymph nodes for the Baumann score relative to the Hartmann score is probably related to the fact that for this method the exact size in mm of lymph nodes has to be measured which is challenging in the absence of contrast. Other studies report different results: Bates et al. described enlarged lymph nodes in only one out of thirteen GLILD

TABLE 3 | Component and composite scores as a percentage of the maximum Baumann and Hartmann score.

Component or composite score	Median (%)		Interquartile range (%)		Minimum-maximum (%)	
	Baumann	Hartmann	Baumann	Hartmann	Baumann	Hartmann
Airway disease	17	6	8–30	2–9	0–65	0–44
Bronchiectasis	25	6	8–42	1–11	0–100	0–68
Bronchial wall thickening	22	4	0–44	0–7	0–83	0–49
Mucus plugging	4	6	0–33	0–11	0–67	0–50
GLILD	40	20	20–40	11–31	0–100	0–63
Nodules	22	28	6–56	6–53	0–100	0–100
Reticulation	50	11	0–83	3–17	0–100	0–42
GGO	67	17	33–100	6–33	0–100	0–78
Total disease	21	9	14–28	6–13	0–56	0–32

Component scores of most common abnormalities and the composite scores of airway disease (sum of bronchiectasis, airway wall thickening, and mucus plugging), granulomatous lymphocytic interstitial lung disease (GLILD) [sum of nodules, reticulation and ground-glass opacities (GGO)] and total disease (sum of all component scores) for both Baumann and Hartmann scoring methods are presented as the median, interquartile range, and total range.

patients (9), while Torigian et al. described enlarged lymph nodes in all five included patients (11).

Although bronchiectasis is not a feature of GLILD, it was the most common CT abnormality, present in 82% of GLILD patients. This result substantially exceeds previously published findings by Torigian (20%), Hartono (35%), Bates (46%), Bouvry (65%), and Mannina (41% diffuse bronchiectasis, 59% focal) (9, 11, 12, 32, 37). Importantly, the patients in some of these studies were younger (9, 11, 32), and in some studies, the interval between time of diagnosis and the CT scan acquisition was shorter (12, 37) Furthermore, the studies by Hartono and Bates did not use scoring methods to analyse the CT scans systematically, which may have led to the underdiagnosis of bronchiectasis. Based on these findings, CVID patients with GLILD have a higher risk of airway disease compared to the risk previously reported for the general CVID cohort (13–16, 25, 38, 39).

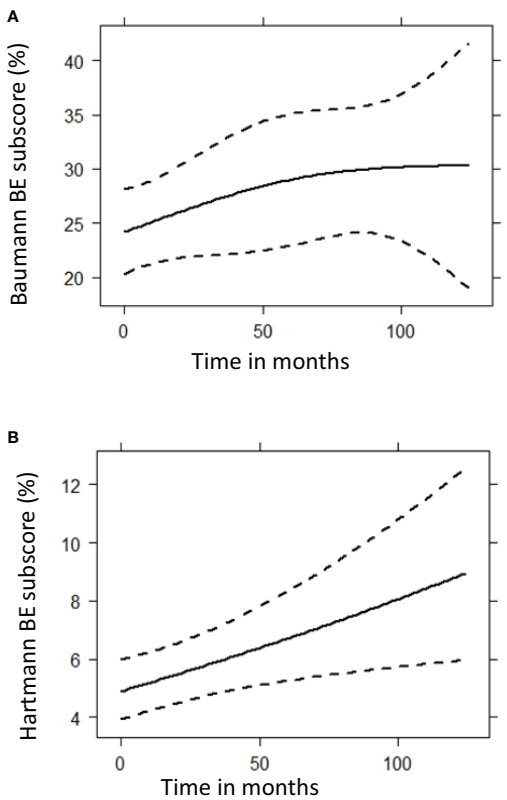

FIGURE 6 | Prediction plots of bronchiectasis (BE) component scores from mixed-effects model analysis. These graphs show the predicted progression in computed tomography BE component scores (%) over time (months) for the Baumann **(A)** and Hartmann **(B)** scoring method, using mixed model analysis. A total of 299 CT scans were used for this follow up analysis. The Baumann BE component score showed no significant change over time ($p = 0.1248$), while the squared root of Hartmann BE score increased significantly ($p = 0.0097$). **(A)** Baumann BE component score. **(B)** Hartmann BE component score.

Longitudinal Analysis

Longitudinal follow-up analysis of 299 CT scans from 81 patients showed that only the Hartmann bronchiectasis component scores increased significantly over time. No increase was observed for the composite scores of GLILD, airway disease or total disease. When interpreting the longitudinal data, it is important to consider that we did not correct for any treatment that was given to the patient, and that it is likely that treatment affects the amount of structural lung disease. In a longitudinal study of 54 CVID patients, scores for bronchiectasis and linear and/or irregular opacities were found to significantly decrease while nodules and GGO did not change (14). Conversely, in another study 14 out of 20 CVID patients exhibited worsening of parenchymal changes on their follow up CT scan (13). However, it should be noted that CT scoring was less standardized and statistical analyses were not performed in this study. Maglione et al. presented CVID cases with waxing-and-waning CT features of ILD over time (5).

To study the natural course of disease progression of GLILD, a cohort study involving the routine acquisition of CT scans is required. Importantly, the risk benefit ratio of such a monitoring strategy is warranted as the radiation exposure needed for chest CT is low and taking into account the considerable morbidity and mortality in GLILD patients. Lung volume, CT protocols, and reconstruction kernels should be standardized, in order to improve the diagnostic yield of each CT scan and allow more sensitive monitoring of disease progression (40–42).

TABLE 4 | Intraclass correlation coefficients for inter- and intra- observer agreement.

Component and composite scores	Intra-observer 1		Intra-observer 2		Inter-observer	
	Baumann	Hartmann	Baumann	Hartmann	Baumann	Hartmann
GLILD (GGO + NOD + RET)	0.88	0.90	0.85	0.85	0.74	0.84
AD (BE + BWT + MP)	0.48	0.78	0.72	0.76	0.42	0.69
Nodules	0.93	0.90	0.86	0.79	0.78	0.85
Bronchiectasis	0.42	0.63	0.78	0.82	0.53	0.66
Reticulation	0.57	0.66	0.61	0.83	0.47	0.38
Bronchial wall thickening	0.55	0.72	0.45	0.47	0.34	0.49
GGO	0.60	0.38	0.86	0.83	0.44	0.35
Consolidation	0.89	0.33	0.73	0.77	0.55	0.72
Mucus plugging	0.48	0.42	0.52	0.57	0.05	0.38

Inter- and inter-observer agreement expressed as the intraclass coefficient values are presented in this table. Intraclass correlation coefficients were defined as follows: 0–0.39 poor, 0.4–0.59 fair, 0.6–0.74 good, and >0.75 excellent (31). GLILD, Granulomatous lymphocytic interstitial lung disease; GGO, ground-glass opacities; NOD, nodules; RET, reticulation; AD, Airway disease; BE, bronchiectasis; BWT, bronchial wall thickening; MP, mucus plugging.

Comparison of Scoring Methods

In this study, two independent CT scoring methods were used to assess GLILD. Baumann scores (**Table 3**) were generally higher, related to the methodology how abnormalities are scored. For example, to compute bronchiectasis component scores for the Baumann method only the most bronchiectatic airways are included. Conversely, to compute bronchiectasis component scores for the Hartmann method also the mean severity of bronchiectasis is included. Consequently, the Baumann method results in higher scores whereas the Hartmann score are in a lower range. Hence, it is not possible to compare the component scores of both methods one-to-one. Longitudinally, the Hartmann method seemed to be more sensitive in assessing bronchiectasis progression over time compared to the Baumann method. The Hartmann method is performed in a lobe-specific manner. Because the Hartmann method provides more precise information about the extent and distribution of lung abnormalities than the Baumann method, this method is more suitable for clinical studies. However, in daily clinical care where time is a limiting factor, the Baumann method might be more feasible to implement.

The Hartmann method also had a slightly higher rate of reproducibility than the Baumann method. The observer agreement for the component score GGO was relatively low for both methods, which might reflect the severe nature of lung disease in GLILD patients: in cases of severe lung disease, the presence and extent of GGO might be harder to assess. Due to the retrospective nature of our study, it is likely that the variable quality of CT scans and reconstruction protocols had a negative impact on the ICCs. Especially the component score reticulation produced low ICCs, which indicates that not all component scores are suitable to monitor GLILD lung disease. Two scoring items exclusive of the Baumann method performed very poor in our study: the subtype of GGO (inflammatory or fibrotic) and subtype of reticulation (inflammatory, fibrotic, or mixed). Thus, these items failed to provide reliable information and to our opinion their relevance is debatable.

However, the component score nodules showed excellent ICCs, and furthermore, the GLILD composite score produced good (Baumann) and excellent (Hartmann) ICCs. A suggestion is to proceed with such scores as main outcomes, while further investigating and improving scoring items with lower reproducibility. Once the relevant changes are agreed upon it will be of interest to transfer the analysis to computer based image analysis in order to render such a scoring method also feasible in regard to time. For this purpose this collection of CT scans will be an excellent resource (43).

CONCLUSIONS

As CT morphology is the one of the major parameters for evaluation during the follow up of GLILD in CVID patients, reliable scoring methods for the longitudinal comparison of interstitial lung changes are required. In this study, we established and evaluated two scoring methods with CT scans of 138 GLILD patients. The composite score for GLILD showed high reproducibility especially according to the Hartmann score, and may become a valuable tool for monitoring disease in longitudinal studies. Once the clinical value of such a score has been demonstrated, automated image analysis systems are needed to optimise the assessment of GLILD and render it suitable for routine diagnostics.

DATA AVAILABILITY STATEMENT

The datasets, i.e. the CT scores and statistics, presented in this article are not readily available. Proposals may be submitted up to 24 months following article publication. To gain access, requestors will need to sign a data access agreement. After 24 months, the data will be available in the data warehouse of the Erasmus university Rotterdam but without investigator support other than the access to the deposited metadata. Requests to access the datasets should be directed to HT (h.tiddens@erasmusmc.nl) and KW (klaus.warnatz@uniklinik-freiburg.de).

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the local ethics committee of the University of Freiburg in Freiburg, Germany (IRB: 189/12), and the national ethical review boards of all participating centers. The patients/

participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

JM: analysis of CT scans, statistical analysis, and first drafts of manuscript. IH: design of Hartmann method and providing training sessions. SG and KW: design and lead of STILPAD. UB: design of Baumann method. AU and MK: collection of CT scans. HT: design of CT study and first drafts of manuscript. E-RA: statistical analysis. All authors contributed to the article and approved the submitted version.

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Institutional affiliations:
[1]Department of Clinical Immunology, Papworth Everard, Royal Papworth Hospital NHS Foundation Trust, Cambridge, United Kingdom
[2]Bircham Park Offices Research Office, Plymouth Hospital NHS Trust, Plymouth, United Kingdom
[3]Department of Clinical Immunology, Nouvel Hôpital Civil, Hôpitaux Universitaires de Strasbourg, Strasbourg, France
[4]Department of Immunology, The Royal London Hospital, Barts Health NHS Trust, United Kingdom
[5]West Midlands Immunodeficiency Centre, Heartlands Hospital, University Hospitals Birmingham NHS Foundation Trust, Birmingham, United Kingdom
[6]Clinic for Immunopathology, Hospital Saint-Louis, Paris, France
[7]Department of Clinical Immunology, John Radcliffe Hospital, Oxford, United Kingdom
[8]Unit for Rheumatology, University Hospital of Munich (LMU), München, Germany
[9]Department of Medicine II (Rheumatology/Immunology), University Hospital of Würzburg, Würzburg, Germany
[10]Clinical Immunology, Royal Free Hospital, Royal Free London NHS Foundation Trust, London, United Kingdom
[11]Department of Internal Medicine, Hospital Haut-Lévêque, CHU de Bordeaux, Pessac, France
[12]Clinic of Immunology and Rheumatology, Hannover Medical School (MHH), Hannover, Germany

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2020.589148/full#supplementary-material

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Histology of Interstitial Lung Disease in Common Variable Immune Deficiency

Fatima Dhalla[1,2†], *Dylan J. Mac Lochlainn*[1†], *Helen Chapel*[1,3*] *and Smita Y. Patel*[1,3]

[1] Department of Clinical Immunology, John Radcliffe Hospital, Oxford University Hospitals NHS Foundation Trust, Oxford, United Kingdom, [2] Developmental Immunology, MRC Weatherall Institute of Molecular Medicine, University of Oxford, Oxford, United Kingdom, [3] Primary Immunodeficiency Unit, Nuffield Department of Medicine and National Institute for Health Research Oxford Biomedical Research Centre, University of Oxford, Oxford, United Kingdom

Interstitial lung disease (ILD) is an important non-infectious complication in several primary immune deficiencies. In common variable immune deficiency (CVID) it is associated with complex clinical phenotypes and adverse outcomes. The histology of ILD in CVID is heterogeneous and mixed patterns are frequently observed within a single biopsy, including non-necrotising granulomatous inflammation, lymphoid interstitial pneumonitis, lymphoid hyperplasia, follicular bronchiolitis, organizing pneumonia, and interstitial fibrosis; ILD has to be differentiated from lymphoma. The term granulomatous-lymphocytic interstitial lung disease (GLILD), coined to describe the histopathological findings within the lungs of patients with CVID with or without multisystem granulomata, is somewhat controversial as pulmonary granulomata are not always present on histology and the nature of infiltrating lymphocytes is variable. In this mini review we summarize the literature on the histology of CVID-related ILD and discuss some of the factors that may contribute to the inter- and intra-patient variability in the histological patterns reported. Finally, we highlight areas for future development. In particular, there is a need for standardization of histological assessments and reporting, together with a better understanding of the immunopathogenesis of CVID-related ILD to resolve the apparent heterogeneity of ILD in this setting and guide the selection of rational targeted therapies in different patients.

Keywords: common variable immune deficiency, interstitial lung disease, histology review, literature analysis, primary immune deficiencies

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***Correspondence:**
Helen Chapel
helen.chapel@ndm.ox.ac.uk

†These authors have contributed equally to this work

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INTRODUCTION

Common variable immune deficiency (CVID) is the most common of the primary immunodeficiency (PID) syndromes with a prevalence of 1 in 25,000 and 50,000, depending on the population (1, 2). It is characterized by low serum levels of IgG, IgA, and/or IgM, and poor specific antibody production (3). There is no definitive diagnostic test, so diagnosis requires the exclusion of secondary hypogammaglobulinaemia, combined immune defects, and, where appropriate, Mendelian disorders (4, 5). Up to 70% of patients suffer with variable non-infectious complications reflecting broader immune dysregulation, including autoimmunity, most commonly autoimmune cytopaenias; lymphocytic infiltration and/or granulomatous inflammation

which can affect the lungs, gastrointestinal tract, spleen, skin or liver; or malignancy, in particular lymphoma (6, 7). Importantly, while bacterial infections are significantly reduced by adequate replacement therapeutic IgG, disease-related complications are not, but are associated with substantially increased mortality (7–9).

Respiratory tract pathology is a major contributor to impaired quality-of-life (10). Bacterial sinopulmonary infections are often the presenting feature, most frequently caused by *Haemophilus influenzae* or *Streptococcus pneumoniae* (11, 12). Recurrent and/or severe lower respiratory tract infections, particularly pneumonia, lead to bronchiectasis with an overall estimated prevalence of 30-35% among CVID patients, which, when present in isolation, does not contribute to increased mortality (8, 11–14). Interstitial lung disease (ILD), on the other hand, probably occurs due to immune dysregulation and/or viral infection rather than as a consequence of bacterial infection (7, 15, 16), and occurs alongside other disease-related complications, and shortens survival (7–9, 16). More rarely, the lungs can be the location for extranodal lymphomas, particularly B-cell non-Hodgkin's lymphomas or MALToma (7, 17–20).

INTERSTITIAL LUNG DISEASE IN COMMON VARIABLE IMMUNE DEFICIENCY

Clinical Significance of CVID-Related ILD

ILD is among the more frequent non-infectious complications of CVID, reported in 15%–60% of patients (7, 9, 14, 21–23). Clinical symptoms and high-resolution computed tomography (HRCT) findings of ILD can appear before or after CVID diagnosis (24, 25). The pathogenesis of CVID-related ILD is presumed to be unrelated to bacterial infections because it can be seen in the absence of bronchiectasis and is not significantly associated with a history of pneumonia (21). Patients with ILD have distinct clinical and immunological phenotypes in keeping with immune dysregulation, in contrast to those without ILD or those with bronchiectasis alone (6, 9, 14, 16, 21, 26, 27). Furthermore, there is no current histological or molecular evidence for chronic bacterial, EBV or CMV viral infections as triggers for inflammation (16, 28–30), though granulomas in other PIDs, such as those with DNA repair defects, show evidence of vaccine derived rubella virus (31). Other related complications, including splenomegaly, autoimmune cytopaenias, persistent lymphadenopathy and lymphoproliferation, but not necessarily granulomata, occur more frequently in patients with CVID-related ILD, supporting at least a role for intrinsic immune dysregulation driving these varied features (6, 9, 16, 21, 27, 32, 33).

Since CVID-related ILD causes significant morbidity, can be progressive and contributes to mortality, there is urgent need for effective treatments (8, 9, 34, 35). Because the mechanism(s) underlying CVID ILD have not been elucidated, immunosuppressive treatments have been tried with varying success, including corticosteroids, ciclosporin, methotrexate, sirolimus, cyclophosphamide, hydroxychloroquine, anti-TNF agents, mycophenolate mofetil, abatacept, rituximab and azathioprine (16, 34, 36–38). Corticosteroids are often used first-line, however, response may be short-lived or incomplete, there are significant side effects associated with protracted use and a proportion of patients are refractory (16, 34, 36, 39). Success with Rituximab, both in combination with azathioprine or mycophenolate mofetil, and as monotherapy, has been reported although controlled trials and long-term outcome data are lacking (40–43). Elevated levels of B-cell activating factor (BAFF), a cytokine that promotes the maturation and survival of B-cells, within the serum and lungs of patients with CVID-related ILD levels drives B-cell hyperplasia and may account for disease progression in a small proportion of patients (15) with invasive B cells in inappropriate germinal centers (28, 44).

Nomenclature

Various terminologies are used for CVID-related ILD, reflecting a lack of consensus regarding the naming of this complication and its heterogeneous nature (45). Lymphoid interstitial pneumonitis was first reported in patients with antibody deficiency in 1973 (46). Since then, various histopathological entities have been reported within lung biopsies of CVID ILD patients, from those caused by polyclonal lymphocytic inflammation to well-formed granulomata, organizing pneumonia, or pulmonary fibrosis, often with mixed pathology within individual patient biopsies (7, 9, 16, 27, 33, 35, 44). "Granulomatous-lymphocytic interstitial lung disease" (GLILD), first proposed in 2004, is often used as an overarching term to describe CVID ILD with lymphocytic infiltrates and/or granulomata (9, 45). However, the accuracy of this term has been called into question. Since not all patients have pulmonary granulomata, it does not fully capture the heterogeneity of the histopathology and similar histological patterns fulfilling a GLILD diagnosis are found in non-CVID PIDs (33, 47).

Investigations for CVID-Related ILD

Non-invasive investigations for CVID-related ILD include elevated serum IgM, decreased class-switched memory B-cells and absolute/relative numerical abnormalities of T-cell populations (15, 16, 34, 35, 48). Alongside rising IgM levels, BAFF, soluble IL-2 receptor and β2microgloblin have also been proposed as serum biomarkers for disease activity (15, 34, 49). Lung function tests, particularly the diffusion capacity for carbon monoxide (DLCO), are useful in monitoring for disease progression and response to treatment, but can lack the sensitivity required for diagnosis, particularly early in the disease course (14, 28, 34, 35, 37). HRCT is highly sensitive for the detection of CVID ILD, including at an early stage before symptoms or abnormal pulmonary function have developed (14, 33, 34). Radiographic findings are mixed and include lymphadenopathy, ground glass opacification, nodularity, septal thickening and consolidation (21, 33, 50). The use of CT combined with positron emission technology (PET) has also been reported as useful to identify sites of active disease, guide biopsy sampling, and monitor response to treatment (41). In selected cases, particularly, but not restricted to, pediatric presentations, genetic testing may be warranted. For example, patients with mutations in *CTLA4*, *LRBA, TACI, KMT2D, XIAP, RAG1*, and *NFKB1* have been found within so called "CVID"

cohorts, and ILD is a common feature of other monogenic PIDs (34, 39, 51–57). A molecular diagnosis enables other therapeutic approaches such as CTLA-4 fusion proteins abatacept and belatacept for the inflammatory associations of CTLA-4 and LRBA deficiency (58, 59). Invasive investigations include assessment of bronchoalveolar lavage fluid for infection and lymphocyte phenotyping, often used to avoid possible complications of biopsy (60), or biopsy of lung tissue under imaging for histopathological assessment.

Importance of Histopathological Assessment of Lung Tissue

Histological assessment of affected lung tissue is essential if features of ILD are present on HRCT. Imaging alone is not sufficient because radiographic patterns of parenchymal lung disease do not correlate with pathological features (33). It has been suggested that tissue from more accessible organs could be used in lieu of lung biopsy (34); however, patients with granulomata at other sites do not necessarily display granulomata within areas of ILD, indicating that other organs do not necessary serve as a proxy for the lung (33). Importantly, histological assessment contributes to the exclusion of differential diagnoses including infection and lymphoma and can provide prognostic information, since interstitial fibrosis has been associated with poorer outcomes (7, 17–20, 33). Currently, it is common practice to subject lung biopsy specimens to hematoxylin and eosin (H&E) staining, immunohistochemical staining for CD3, CD4, CD20/19 and EBV and CMV viral infections (37, 44). Understanding the pathological processes at play and the phenotype of infiltrating immune cells can help rationalize the selection of therapeutics used for CVID ILD (40–43).

We have reviewed the published literature of large series (>10 cases) for detailed histological findings of CVID ILD, the most recent being Larsen et al. (46). It is not always possible to know which patients were included in previous reports so only the most recent from each center is used unless marked (**Table 1**). Variations including the methods used for both biopsy and reporting are discussed in Section 4.

HISTOLOGICAL PATTERNS OF ILD IN CVID

The histological abnormalities reported in CVID ILD vary and overlap extensively. Similar patterns can also be found in numerous other lung diseases, making diagnosis challenging (44). Using a similar structure as Rao et al. (44), we summarize the commonly reported lung biopsy findings, each of which we discuss in turn (**Table 1**).

Granulomata

The granulomata reported in CVID ILD can vary from poorly- to well-circumscribed, with an apparent predilection for the former (28, 33, 44). Non-infectious CVID granulomatous lung disease shares some similar histological features with sarcoidosis and hypersensitivity pneumonitis; thus, clinical and radiological correlation is important in distinguishing these conditions (44, 62). "Poorly-formed granulomata" have been found within areas of pulmonary lymphoid hyperplasia and are difficult to define, as these are very subjective; additionally, granulomata can be found throughout the lung parenchyma (28, 44). It is worth re-emphasizing that granulomata are not reported in all cases of CVID-related ILD, with frequencies ranging from 0-94% depending on the individual study (**Table 1**) (7, 33, 44, 47). This suggests that there may be more than one pathological process in CVID-ILD (33, 47) and that the generalized use of overarching term "GLILD" to refer to all CVID-related ILD can be misleading.

Pulmonary Lymphoid Hyperplasia

Lymphoid proliferation has been designated as the "cardinal" feature of CVID ILD, and different patterns of pulmonary lymphoid hyperplasia (PLH) have been described, including follicular bronchiolitis, lymphocytic interstitial pneumonitis (LIP), lymphocytic infiltrates, and nodular lymphoid hyperplasia (28, 38, 40, 44, 47). In one case series where severity was assessed, PLH tended to be toward the moderate to severe end of the spectrum,

TABLE 1 | Histological lung biopsy findings from common variable immune deficiency (CVID) patients reported in the literature.

			Histological findings						
Publication (Ref)	**Number of CVID patients with lung biopsies**	**Granulomata n (%)**	**Pulmonary Lymphoid Hyperplasia**				**Organizing pneumonia**	**Pulmonary Fibrosis**	
			Interstitial inflammation	***(Peri)bronchial inflammation***	***Lymphocytic infiltration***	***Lymphoid hyperplasia***		***Fibrosis***	***Remodeling***
Rao et al.* (44)	16	15 (93%)	16 (100%)	16 (100%)	NS	NS	14 (87%)	12 (75%)	6 (37%)
Patel et al. (33)	19	1 (5%)	11 (58%)	7 (37%)	15 (79%)	NS	6 (32%)	8 (42%)	3 (16%)
Maglione et al. (21)	12	3 (25%)	4 (33%)	4 (33%)	2 (17%)	4 (33%)	4 (33%)	4 (33%)	NS
Larsen et al. (47)	34	23 (68%)	12 (35%)	22 (65%)	NS	10 (29%)	25 (71%)	1 (3%)	NS
Verbsky et al.* (61)	34	31/34 (91%)	NS	33/34 (97%)	33/34 (97%)	NS	30/34 (88%s	13/34 (32%)**	NS

*Only publications with sufficient histological detail were included; single case histories or small studies (less than 10) are not included. Rao et al. (44) and Patel et al. (33) reported their findings in similar terms, but these varied in other publications. Efforts were made to group similar findings on the basis on similar histological terms in these instances. Where detail for a given finding was not specified (NS), this is also indicated. *Where the inclusion of previously published cases in a paper could not be completely excluded. ** on CT not reported on histology.*

with peribronchiolar and interstitial lymphocytic inflammation (44). These patterns often occur together and are rarely found in isolation (33, 44). Follicular bronchiolitis and/or LIP are found in around half of the cases reviewed (**Table 1**), and this is also in keeping with a recent review where 20/46 patients had some form of lymphoid infiltration, though not always specified (7).

Organizing Pneumonia

Organizing pneumonia (OP), intra-alveolar buds of granulation tissue with myofibroblasts and connective tissue, is reported in a substantial number of histological specimens, although to varying degrees between studies (**Table 1**). Cryptogenic organizing pneumonia (COP) is also found in CVID patients and is an important differential diagnosis when OP is the predominant finding on biopsy (40, 44). However, Rao et al. demonstrated the potential for misdiagnosis of CVID ILD when isolated COP was found on limited biopsy samples obtained by bronchoscopy.

OP can have many aetiologies. Larsen et al. reported that in their cohort OP was accompanied by a "dense lymphoid infiltrate", which was not seen in biopsies from other causes of OP (47). Therefore, in their cohort of 34 patients with CVID and 4 with IgAD, these authors suggest that the combination of these two findings should suggest CVID or IgA deficiency rather than another etiology.

The lack of overlap between OP and pulmonary fibrosis (1/19 cases) in our cases might indicate separate pathological entities; however, significant overlap was described by Rao et al. (11/16 cases) (33, 44), who suggested evolving pathology.

Pulmonary Fibrosis

Pulmonary fibrosis is described in a quarter of CVID ILD cases (**Table 1**); however, similar to OP, one case series accounts for most of these cases (44), where the majority of patients had some degree of fibrosis. In contrast, Ho et al. found 6.3% of cases where "extensive pulmonary fibrosis" was the "predominant" finding at the time of biopsy; however, it was not reported whether it was a feature in other biopsies to a lesser degree (7).

Interstitial fibrosis in CVID ILD together with lymphoproliferation may resemble some of the patterns of idiopathic interstitial pneumonia, particularly if significant fibrosis (44). Only two studies looked specifically for architectural remodeling, and one of these found this to be associated with significant interstitial fibrosis (33, 44). The presence of fibrosis is a poor prognostic factor; prospective clinical studies are needed to justify earlier treatment (33).

Immunohistochemistry

Immunohistochemical staining of the lymphocytic infiltrate has produced discordant findings in the cases where it has been performed. $CD20^+$ B-cells were found in a small proportion of cases, in follicles with T-cells circumscribing them, although T-cells are also reported more diffusely and in areas without B-cells (28, 33, 44). Rao et al. found a predominance of $CD4^+$ T-cells within lymphoid infiltrates and also observed the presence of B-cell follicles surrounded by $CD4^+$ T-cells (44). We recently reported a predominance of T-cells in most cases (**Figure 1A**), either $CD4^+$ or $CD8^+$; only 1 of six had germinal centers within B-cell follicles (**Figure 1B**) (33). Maglione et al. reported actively proliferating germinal centers in some of their patients with B-cell follicles (28). It is important to differentiate these from pulmonary MALToma, as found in two patients in the Oxford series (33).

We suggested that since the predominant T-cells were either $CD4^+$ or $CD8^+$, this pointed to different pathological entities (33). Chase et al. hypothesized that the inflammatory infiltrate, including B- and T-cells, might contribute to progressive ILD and pulmonary fibrosis, something that therapy directed against B- and T-cells might possibly prevent (40). Similarly, Maglione et al. suggested B-cells may be responsible for leukocyte accumulation in their role as antigen presenting cells and producers of chemokines and/or cytokines, making them a therapeutic target (28).

ADDRESSING THE HETEROGENEITY OF HISTOPATHOLOGICAL FINDINGS CVID-RELATED ILD

There is a large amount of histopathological heterogeneity in biopsies from CVID-related ILD cases, both from one patient to the next, as well as between different case reports (**Table 1**). We discuss possible reasons for this in respect to the underlying pathophysiology, the patient populations reported, and factors relating to obtaining and interpreting lung biopsies.

Pathophysiology: A Spectrum of Disease, Separate Diseases, or a Shared Endpoint for Several Diseases?

Since the pathophysiology of CVID ILD is unknown, it is not surprising that there is no explanation for the degree of heterogeneity in the histology (33, 44). CVID-related ILD (or GLILD) was originally defined as a "conglomeration of pulmonary histopathologic abnormalities seen in a subset of patients with CVID (44). The divergent findings may represent a "spectrum" of a single disease (44) or several different pathologies, in addition to the primary antibody deficiency. Another hypothesis is that CVID ILD represents a common "pulmonary reaction pattern" (or "morphological common endpoint") not only for CVID but also for other PIDs in which similar clinical, radiographical, and histological features have been described (44, 47). None of these hypotheses are mutually exclusive; it may be that the small numbers and the absence of international standardization frustrate the recognition of distinct pathological patterns.

Patient Populations

Geography may influence the variability observed, with different genetic influences in particular populations. It is interesting that three of the large CVID-related ILD case series, one from the UK and two from the USA, show the most divergence, despite a conscious effort on the part of the former to adhere to similar definitions used previously.

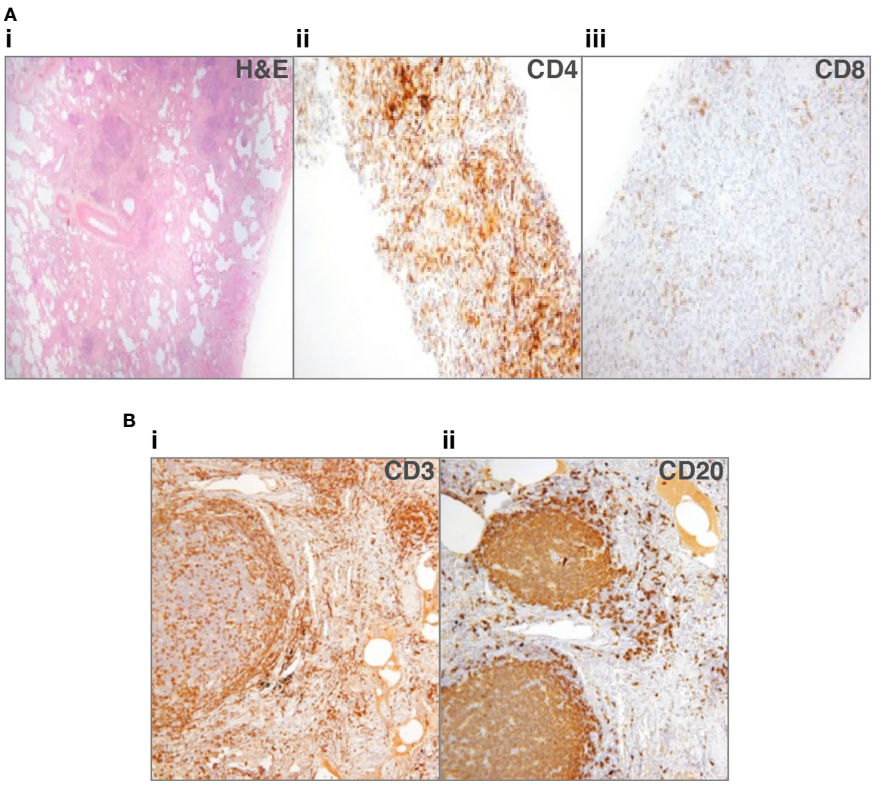

FIGURE 1 | Lung biopsies from patients with common variable immune deficiency (CVID)-related interstitial lung disease (ILD). **(A)** Patient 1: (i) lung biopsy section stained with hematoxylin and eosin (H&E), to show lack of alveolar spaces, and many lymphocytes infiltrating the interstitium (ii) shows staining for $CD4^+$ cells that predominate, sometimes in nodules, (iii) shows scanty $CD8^+$ cells (33). No granulomata or organizing pneumonia. **(B)** Patient 2: (i) lung biopsy section stained for $CD3^+$ cells, showing that T-cells surround follicles and are additionally found in discreet nodules, (ii) shows the follicles to consist of $CD20^+$ cells, with only scattered $CD20^+$ B-cells in other areas. No granulomata or organizing pneumonia.

Differences in clinical practice, including diagnosis, cannot be totally discounted. Some series are restricted to patients with spontaneous (non-familial) CVID in adults and others include patients diagnosed in childhood. Since no diagnostic details are given, the exclusion of combined immune deficiencies involving T-cell immunity as well as B-cell failure (5), or known mutations in monogenic disease (e.g. *CTLA4, LRBA, KMT2D, XIAP, RAG1, NFKB1*) (34, 39, 51–57, 63) is unclear.

Biopsy-Related Factors: Technique, Timing, Treatment, and Interpretation

The method by which a biopsy has been obtained may have a significant impact on the clinical conclusions reached (61). Given that several different biopsy techniques have been used across the cases reported, this may be a contributing factor to some of the variation between cases, though in almost all series so far, imaging was used to obtain the biopsy.

A further consideration is the timing of the biopsy with respect to disease progression but most patients do not undergo repeat biopsies. It is likely that once pulmonary fibrosis and possibly organizing pneumonia are present that these may progress (33).

Another potential contributing factor is whether the biopsy was performed prior to or following corticosteroid or immunosuppressive treatment. These drugs could plausibly alter the patterns observed or mask them entirely, particularly those related to inflammation. While some authors have clearly documented when such drugs were used before biopsies were performed (33), this is not always the case, so firm conclusions cannot be drawn.

In the absence of standardized reporting, reading of the biopsy adds a great deal of potential for variation to be introduced. Although some authors have tried to mirror the approach pioneered by others and/or have a second, independent pathologist review the histology, some degree of both intra- and inter-operator variability is inevitable when faced with an uncommonly encountered pathological entity (33, 40).

CONCLUSIONS AND FUTURE DIRECTIONS

In summary, there is considerable heterogeneity in the histopathological findings both within individual patients,

between patients and between study centers, which include lymphoid hyperplasia, granulomata, organizing pneumonia and pulmonary fibrosis. The term "GLILD" is best avoided as not all patients have pulmonary granulomata (32, 46), and its use may mask the histopathological complexity and/or multiple pathological processes (33, 47).

Possible explanations include differences in the timing of sampling with respect to the disease process or treatments, genetic, geographical and environmental factors (7, 33, 44, 47). Finally, inconsistencies in obtaining histological specimens, treated, immuno-stained and described between studies have contributed (33), highlighting an urgent need for standardization of histopathological findings, to allow fairer comparisons to be made between distinct studies. The ability to compare separate studies is of paramount importance when dealing with a rare disease entity.

We need to expand our understanding of the etiology and immunopathogenesis of ILD in CVID, to provide more accurate prognostication and select appropriate treatments. Future studies will incorporate detailed cellular phenotypic, proteomic, transcriptomic and genomic dissection of CVID-ILD, to shed further light on pathogenesis, identify disease-relevant biomarkers and better guide treatment selection.

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FD and DM prepared the first draft of the manuscript. All authors contributed to editing of subsequent versions and reviewed and authorized the final version. HC and SP played a supervisory role. All authors contributed to the article and approved the submitted version.

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Managing Granulomatous–Lymphocytic Interstitial Lung Disease in Common Variable Immunodeficiency Disorders: e-GLILDnet International Clinicians Survey

Annick A. J. M. van de Ven[1†], Tiago M. Alfaro[2†], Alexandra Robinson[3], Ulrich Baumann[4], Anne Bergeron[5], Siobhan O. Burns[6], Alison M. Condliffe[7], Børre Fevang[8], Andrew R. Gennery[9], Filomeen Haerynck[10], Joseph Jacob[3,11], Stephen Jolles[12], Marion Malphettes[13], Véronique Meignin[14], Tomas Milota[15], Joris van Montfrans[16], Antje Prasse[17], Isabella Quinti[18], Elisabetta Renzoni[19], Daiana Stolz[20], Klaus Warnatz[21,22†], John R. Hurst[3†] and on behalf of the ERS e-GLILDnet Clinical Research Collaboration*

[1] Departments of Internal Medicine and Allergology, Rheumatology and Clinical Immunology, University Medical Center Groningen, Netherlands, [2] Pneumology Unit, Centro Hospital e Universitário de Coimbra, Coimbra, Portugal and Centre of Pneumology, Faculty of Medicine, University of Coimbra, Coimbra, Portugal, [3] UCL Respiratory, University College London, London, United Kingdom, [4] Department of Paediatric Pulmonology, Allergy and Neonatology, Hannover Medical School, Hannover, Germany, [5] Université de Paris, Assistance Publique Hôpitaux de Paris (APHP), Hôpital Saint Louis, Paris, France, [6] Institute of Immunity and Transplantation, University College London, Dept of Immunology, Royal Free London NHS Foundation Trust, London, United Kingdom, [7] Department of Infection, Immunity and Cardiovascular Diseases, University of Sheffield Medical School, Sheffield, United Kingdom, [8] Centre for Rare Disorders and Section of Clinical Immunology and Infectious Diseases, Oslo University Hospital, Oslo, Norway, [9] Translational and Clinical Research Institute, Newcastle University and Great North Children's Hospital, Newcastle upon Tyne, United Kingdom, [10] Department of Pediatric Pulmonology and Immunology, Centre for Primary Immune deficiency Ghent, PID research lab, Ghent University Hospital, Belgium, [11] Centre for Medical Image Computing, University College London, London, United Kingdom, [12] Immunodeficiency Centre for Wales, University Hospital of Wales, Cardiff, United Kingdom, [13] Department of Clinical Immunology, Hôpital Saint-Louis, Assistance Publique Hôpitaux de Paris (APHP), Université Paris Diderot, Paris, France, [14] Department of Pathology, Hôpital Saint-Louis, Assistance Publique Hôpitaux de Paris (APHP), Paris, France, [15] Department of Immunology, Second Faculty of Medicine Charles University and Motol University Hospital, Prague, Czech Republic, [16] Department of Pediatric Immunology and Infectious Diseases, University Medical Center Utrecht, Utrecht, Netherlands, [17] Department of Pulmonology, Hannover Medical School and DZL BREATH, and Fraunhofer Institute for Toxicology and Experimental Medicine, Hannover, Germany, [18] Department of Molecular Medicine, Sapienza University of Rome, Rome, Italy, [19] Interstitial Lung Disease Unit, Royal Brompton Hospital, London, United Kingdom, [20] Clinic for Respiratory Medicine and Pulmonary Cell Research, University Hospital Basel, Basel, Switzerland, [21] Department of Rheumatology and Clinical Immunology, Medical Center - University of Freiburg, Faculty of Medicine, University of Freiburg, Freiburg, Germany, [22] Center for Chronic Immunodeficiency, Medical Center - University of Freiburg, Faculty of Medicine, University of Freiburg, Freiburg, Germany

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Guzide Aksu,
Ege University, Turkey

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University of South Florida, United States

***Correspondence:**
John R. Hurst
j.hurst@ucl.ac.uk

[†]These authors have contributed equally to this work

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Background: Granulomatous–lymphocytic interstitial lung disease (GLILD) is a rare, potentially severe pulmonary complication of common variable immunodeficiency disorders (CVID). Informative clinical trials and consensus on management are lacking.

Aims: The European GLILD network (e-GLILDnet) aims to describe how GLILD is currently managed in clinical practice and to determine the main uncertainties and unmet needs regarding diagnosis, treatment and follow-up.

Methods: The e-GLILDnet collaborators developed and conducted an online survey facilitated by the European Society for Immunodeficiencies (ESID) and the European Respiratory Society (ERS) between February–April 2020. Results were analyzed using SPSS.

Results: One hundred and sixty-one responses from adult and pediatric pulmonologists and immunologists from 47 countries were analyzed. Respondents treated a median of 27 (interquartile range, IQR 82–maximum 500) CVID patients, of which a median of 5 (IQR 8–max 200) had GLILD. Most respondents experienced difficulties in establishing the diagnosis of GLILD and only 31 (19%) had access to a standardized protocol. There was little uniformity in diagnostic or therapeutic interventions. Fewer than 40% of respondents saw a definite need for biopsy in all cases or performed bronchoalveolar lavage for diagnostics. Sixty-six percent used glucocorticosteroids for remission-induction and 47% for maintenance therapy; azathioprine, rituximab and mycophenolate mofetil were the most frequently prescribed steroid-sparing agents. Pulmonary function tests were the preferred modality for monitoring patients during follow-up.

Conclusions: These data demonstrate an urgent need for clinical studies to provide more evidence for an international consensus regarding management of GLILD. These studies will need to address optimal procedures for definite diagnosis and a better understanding of the pathogenesis of GLILD in order to provide individualized treatment options. Non-availability of well-established standardized protocols risks endangering patients.

Keywords: CVID, GLILD, interstitial lung disease, e-GLILDnet, diagnosis, follow-up, treatment

INTRODUCTION

Common variable immunodeficiency (CVID) disorders are the most prevalent symptomatic primary immunodeficiency (PID) conditions, characterized by hypogammaglobulinemia together with an increased susceptibility to infections and/or, in a minority of patients, clinically significant immune dysregulation (1). Immune dysregulation includes autoimmune and autoinflammatory conditions, lymphoproliferative disease and can result in both solid organ and hematologic malignancies. With generally efficacious administration of immunoglobulin substitution and antimicrobial agents, immune dysregulation now imposes the heaviest burden on morbidity and mortality of CVID patients. The term "CVID" was in 2009 redefined by the International Union of Immunological Societies Expert Primary Immunodeficiency Committee into "CVID disorders", emphasizing the heterogeneity of this collection of inborn errors of immunity (2). The number of potential distinct entities within this group remains unknown and although novel monogenic forms are still being identified, the majority of cases is assumed to be of complex and polygenic inheritance (3, 4).

Lung involvement is very common in CVID disorders and typically has two not mutually exclusive entities: structural abnormalities such as bronchial wall thickening, air trapping and bronchiectasis that can arise as complications of recurrent bronchopulmonary infections; and interstitial lung disease (ILD) including parenchymal and interstitial abnormalities (ground glass opacities, nodules and consolidation) that are considered to be driven by intrinsic CVID-related immune dysregulation. This ILD in CVID disorders is commonly referred to as granulomatous-lymphocytic interstitial lung disease or GLILD. The estimated prevalence of GLILD in CVID disorders is around 15% and may already be present in childhood CVID disorders (5–7).

GLILD was defined by a UK Consortium as "a distinct clinico-radio-pathological ILD occurring in patients with CVID disorders, associated with a lymphocytic infiltrate and/or granuloma in the lung, and in whom other conditions have been considered and where possible excluded", recognizing that this GLILD is "usually seen in the context of multisystem granulomatous/inflammatory involvement" (8). This definition of GLILD was unanimously supported by all participants. Agreement scores on other aspects of GLILD diagnosis were lower: for instance, 47% agreed that GLILD patients need to be symptomatic. The report went on to describe that diagnostic evaluation should include spirometry (96% consensus), lung volumes (91%), gas transfer (100%), flexible bronchoscopy to exclude infection (83%), surgical lung biopsy (83%) and computed tomography (CT, all respondents). Consensus was defined that lung biopsy specimens should be stained for CD3, CD4, CD8, CD20, for the presence of bacteria including *Mycobacteria* and for fungi, and for clonality to exclude lymphoma (8).

The pathogenesis of GLILD remains unclear and is considered to be heterogeneous. Histologic studies reveal

infiltration of both T- and B-lymphocytes, partly leading to the formation of tertiary lymphoid structures. Increased concentrations of local and serum B-cell activating factor (BAFF) possibly drive B-lymphocyte hyperplasia (9).

GLILD is a rare condition and therefore there is a lack of robust scientific evidence, especially about therapeutics. There are currently no published randomized controlled trials or prospective cohort studies investigating the effects of immunomodulatory treatments as many difficulties arise in recruiting an adequate number of participants. A systematic review is included in this collection (Lamers et al., this issue). Current investigations are exclusively observational studies; this is problematic as they are unable, by design, to include randomization and concealment of allocation (10).

The first step in GLILD treatment consists of optimization of CVID disorders management, including Ig replacement therapy (IgRT). Antimicrobial prophylaxis may be used in a proportion of patients, with initiation of immunosuppressive therapy given that IgRT alone is not generally effective to treat GLILD (11–15). As with many inflammatory conditions, corticosteroids are often the first choice for remission induction in GLILD. Corticosteroids often result in an improvement in GLILD, however following prednisolone therapy of 1–4 months a widely heterogeneous response was observed, as many patients do not exhibit any improvements in PFTs or had disease flares upon tapering of corticosteroid medication (16). Collectively, these findings define the need for re-evaluation of corticosteroid monotherapy as first-line treatment.

Regarding second-line immunosuppressive therapy, various drugs have been employed. Small case series (17) and single case reports (18–21) show a potential effect of rituximab as monotherapy. Rituximab is also documented to be used in combination with azathioprine (21–28), 6-MP (22, 29) or mycophenolate mofetil (22, 28, 30), supporting a role for B-lymphocytes in the pathogenesis of GLILD. Other therapies include conventional disease modifying anti-rheumatic drugs (cDMARD), such as cyclophosphamide and methotrexate, however, current evidence is limited and lacks scientific support through a lack of controlled clinical trials (31). We are not aware of any reports using novel anti-fibrotics used in fibrotic ILD such as nintedanib and pirfenidone.

The scarcity and low level of quality of scientific literature on GLILD highlights knowledge gaps in essential aspects of GLILD, including pathogenesis, diagnostic evaluation and therapy. Since GLILD is a rare disease, these data can only be obtained by means of constructive, multicenter and multidisciplinary collection and collaboration.

With this aim, the e-GLILDnet was established in 2019 as a Clinical Research Collaboration of the European Respiratory Society (https://www.ersnet.org/research/e-glildnet—a-european-granulomatous-lymphocytic-interstitial-lung-disease-network; twitter: @glildnet) (32). A first workstream of this group was to conduct an online questionnaire among treating physicians of which the results are described here.

METHODS

An online questionnaire was distributed to members of the European Respiratory Society (ERS) and European Society for Immunodeficiencies (ESID) between February 19 and April 30, 2020, and promoted on Social Media.

The questionnaire was developed by the e-GLILDnet collaborators and pretested. Questions were designed by authors with experience in immunology (AV, KW) and pulmonology (TA, JH) and previous experience in designing online questionnaires (KW) (33) Multiple rounds of revision within this author group and subsequently the entire e-GLILDnet team followed. Final adjustments were made after testing an online pilot version. The questionnaire was distributed (in English), and comprised 35 combined open/multiple choice questions focusing on screening, diagnosis, treatment and follow-up of GLILD.

After April 30, 2020, data were collected and categorized for further analysis. Data were transferred and stored in an electronic database of IBM SPSS Statistics (version 23) for Windows, Armonk NY. Statistical analyses consisted of descriptive statistics and comparison of categorical data using Pearson Chi square or Fischer exact tests. A p value of < 0.05 was considered statistically significant.

RESULTS

Clinicians Treating GLILD Rarely Have Access to Standardized Protocols

A total of 161 substantially completed clinician surveys were returned. Responses came from 47 different countries, most frequently Italy (n=17), followed by France, Spain, United Kingdom (each n=14) and Australia, Czech Republic, Germany, Portugal and the U.S.A. (each n=5).

The majority (n=127, 78.9%) of respondents treated adult patients and were specialized in either pulmonology (n=81, 50.3%) or immunology (n=38, 23.6%). Other specialties (n=11, 6.8%) included internal medicine and infectious diseases. The 31 responding pediatricians were specialized in immunology (n=24, 14.9%) or pulmonology (n=7, 4.3%) and two additional respondents treated both adult and pediatric immunology patients. The responses from these two subjects were analyzed in both groups for descriptive statistics but excluded from comparisons between those treating adult and pediatric patients.

Respondents treated a median of 27 (range 0 to 500) CVID disorders patients and 5 (0 to 200) GLILD patients with a large variation between respondents. Only a small proportion (n=11, 6.8%) worked at a secondary care hospital, the majority was employed at specialized settings including tertiary care hospitals (n=82, 50.9%) and/or reference centers for PID/CVID disorders (n=61, 37.9%) or ILD/sarcoidosis (n=56, 34.8%). More pediatricians worked at a PID reference center (58.1% *vs* 33.1%; p = 0.01) and/or in an academic setting (77.4% *vs* 44.9%, p=0.001) than specialists treating adults. Conversely, there were no pediatricians employed at

ILD/sarcoidosis references centers, compared to 44.1% of the adult specialists (p<0.001).

Despite these specialized work environments, only 19.3% of respondents reported the availability of a dedicated GLILD protocol.

The Diagnosis of GLILD Is Often Difficult

When asked about screening for lung disease in CVID disorders patients with no established structural lung disease (i.e. GLILD and/or airway disease), most respondents stated using pulmonary function tests at least once a year (n=110, 70.9%). Chest CT was less frequently used, with 63.2% of respondents using CT for screening in asymptomatic patients at intervals between ≥1–3 years up to every 5–10 years. Immunologists (75.4 *vs* 54.8% for pulmonologists, p=0.008) and those working at PID reference centers (83.3 *vs* 50.5%, p<0.001) reported greater use of CT screening. There were no differences between pediatricians and those caring for adults (64.3 *vs* 63.2%, p=0.548). Nearly all respondents (94.4%) admitted having at least sometimes difficulties diagnosing GLILD, with 38.3% stating that GLILD diagnosis was often difficult. These difficulties were similar between different specialties and centers.

The tests used for the evaluation of suspected GLILD are described in **Table 1**. Whilst not definitive, the majority of clinicians reported using sputum and bronchoalveolar lavage (BAL) tests, and half of them used blood investigations. The use of biopsy was much less frequent: 46 respondents (28.6%) stated that histology is required for diagnosis, but 71.4% would not routinely undertake a biopsy. Respondents were questioned on the results from biopsies from patients with suspicion of GLILD, and 46 (28.9%) out of 103 stated that alternative diagnoses had been found. Elaborating upon these alternative diagnoses, lymphoma was most frequently reported, but malignancy or lung cancer not further specified were also mentioned. Second were infections, including TB, fungal infection and one case of EBV induced lipoid pneumonia. One respondent mentioned hypersensitivity pneumonitis. Furthermore, other conditions mentioned included nonspecific interstitial pneumonia (NSIP), granulomatous diseases, sarcoidosis, lymphoproliferative disorders, post-inflammatory fibrosis and organizing pneumonia which however may be considered part of the spectrum of GLILD.

TABLE 1 | Performed diagnostics in the evaluation of suspected GLILD/ exclusion of other pathology.

	No. (total 161)	Percentage (%)
Blood	**118**	**73.3**
Aspergillus antigen blood test	80	49.7
Mycobacterium blood test	80	49.7
Beta D glucan blood test	41	25.5
Other blood tests*	33	20.5
Sputum	**121**	**75.2**
Bacteria	108	67.1
Mycobacteria	108	67.1
Fungal pathogens	90	55.9
Viral pathogens	43	26.7
Other sputum tests	5	3.1
Bronchoalveolar lavage	**129**	**80.1**
Bacteria	124	77
Mycobacteria	121	75.2
Fungal pathogens	119	73.9
Viral pathogens	87	54
Other bronchoalveolar lavage tests**	39	24.2
Lung biopsy	**39**	**24.2**
Bacteria	22	13.7
Mycobacteria	30	18.6
Fungal pathogens	26	16.1
Viral pathogens	17	10.6
Other biopsy tests	11	6.8

**Other blood tests include culture, autoantibody panel, beta 2 microglobulin, soluble CD25, cytology differential, Igs, procalcitonin, PCR EBV, and CMV. **Other bronchoalveolar lavage tests include next generation sequencing of pathogens, galactomannan, flow cytometry.*

Disparities in Follow-Up and Criteria for Initiation of Immunosuppressive in GLILD

Since there are no clear guidelines on how to carry out follow-up of GLILD patients, we asked whether respondents experienced difficulties in deciding follow-up. This question was filled out by 110 respondents, of which 18 (16.3%) mentioned that they did not experience difficulties at all in defining adequate follow-up for GLILD. The majority however experienced difficulties at different aspects, namely in defining the optimal time interval for follow-up (55.5%), defining the optimal monitoring method (39.1%) and how to follow-up on asymptomatic patients (43.6%) and patients that did not require current treatment (24.5%).

We asked how follow-up of asymptomatic patients not requiring therapy was carried out with regard to monitoring methods and time interval (**Table 2**). The same questions were asked for patients

TABLE 2 | Preferred monitoring time intervals for untreated and treated patients per modality.

	Asymptomatic, untreated GLILD patients		GLILD patients requiring treatment	
	1st choice	2nd choice	1st choice	2nd choice
Clinical and laboratory evaluation	3–4 monthly (n = 46, 40.4%)	6–8 monthly (n = 41, 36%)	3–4 monthly (n = 58, 50.9%)	1–2 monthly (n = 42, 36.8%)
PFT	6–8 monthly (n = 52, 44.8%)	12 monthly (n = 37, 31.9%)	3–4 monthly (n = 67, 58.3%)	6–8 monthly (n = 28, 24.3%)
CXR	12 monthly (n = 28, 26.9%)	6–8 monthly (n = 21, 19.6%)	3–4 monthly (n = 32, 31.4%)	6–8 monthly (n = 14, 13.7%)
HRCT	>12 monthly (n = 61, 53.5%)	12 monthly (n = 40, 35.1%)	6–8 monthly (n = 40, 35,4%)	12 monthly (n = 32, 28,3%)

CXR, chest X-ray; GLILD, granulomatous–lymphocytic interstitial lung disease; HRCT, high-resolution computed tomography; PFT, pulmonary function tests.

that did require current therapy. Questions were filled out by 102–116 respondents. As expected, the selected time intervals were on average shorter than for patients not requiring therapy. Chest X-ray was not considered to be an applicable monitoring method by 35% of the respondents. A different subset of respondents however seemed to value chest X-ray for monitoring patients requiring therapy; of the 66 respondents that used CXR in this group, almost half of them (n=32; of which 22 were adult pulmonologists) applied this modality every 3 to 4 months.

Clear-cut criteria on when to initiate immunosuppressive therapy in GLILD have not been defined and this was reflected in the dissimilar answers given to this question. A diagnosis of GLILD alone was for the majority of respondents (n=82 out of 103, 79.6%) not sufficient reason to start an immunosuppressive treatment regimen. Similarly, the presence of clinical symptoms alone (n=86, 83.5%) or deteriorating PFT (n=80, 77.6%) or HRCT findings (n=77, 74.8%) alone was usually insufficient basis for commencement of therapy. The fraction of respondents that would initiate therapy increased if there were abnormalities in two out of three of the aforementioned items but remained relatively low; (31.1% for clinical symptoms and PFT decline; up to 47.6% for HRCT and PFT deterioration). Strikingly, only 60.2% would treat "All patients with impaired lung function, clinical symptoms and worsening of CT scan". Adult pulmonologists (75%) were most likely to initiate treatment in this patient category, followed by adult immunologists (60%) and pediatricians (12.5%).

Therapy of GLILD: Variable Use of Steroids for Remission-Induction and Maintenance Therapy

The next part of the survey included questions related to the treatment of GLILD. Respondents were questioned whether they had used glucocorticoids for remission induction and/or maintenance therapy in GLILD patients and if so, in how many patients. Of the 125 respondents that filled out this question, 82 (65.6%) had used monotherapy with glucocorticoids for remission induction. This was equally distributed between adult immunologists and pulmonologists. The majority (n=63, 77.8%) had used this regimen in 1–5 patients; ten (12.3%) and eight (9.9%) clinicians had treated 5–10 or >10 patients, respectively. Questions on dosage and tapering revealed that the commonest regimen for severe GLILD was 1mg/kg body weight (BW), as performed by 50%, but 0.5 mg/kg BW was also frequent (32/82 respondents, 39.0%). Only one respondent used a dose lower than 0.5mg/kg BW and some clinicians used more than 1mg/kg BW. The twelve responding pediatricians used significantly higher doses than clinicians treating adults only; six of them used 1mg/kg BW and the other six used >1mg/kg BW ($p<0.001$, Pearson Chi-square). The distribution of the tapering period of glucocorticoids was comparable between groups; most physicians (n=38, 46.3%) tapered glucocorticoids entirely or until maintenance dose within 1–3 months, but longer or more variable intervals were also reported. The experience on effectiveness of this therapy was diverse: only three respondents (3.7%) replied that nearly all patients responded; in general, respondents felt that the majority of (n=47, 58.0%) or some patients (n=27, 33.3%) responded.

The proportion of respondents that used glucocorticoids for maintenance therapy was 58 out of 124 (46.8%). Noticeably, six of them did not report using glucocorticoids for remission induction. Again, patient numbers treated by individual clinicians were small with 1–5 patients for the majority (n=47, 82.5%) of clinicians. About two-third used ≤7.5mg steroids daily and slightly under one-third used 7.6–15mg per day. Three respondents had used maintenance doses >15mg/day. The clinical response to these maintenance glucocorticoids was heterogeneous and many responded with multiple answers; complete and partial responses to maintenance glucocorticoids were noted by 20 and 62.7% of 59 respondents, respectively. A sustained response was seen by 16 (27.1%), but relapses occurred frequently as well (n=20, 33.8%).

Therapy of GLILD: Azathioprine, Rituximab and Mycophenolate Mofetil Are the Most Frequently Employed Steroid-Sparing Agents

Following the questions on glucocorticoid use, respondents were asked on their experience with other immunosuppressive agents for treatment of GLILD. These included both cDMARD, biologicals such as rituximab, TNF inhibitors and combinations of both. Respondents were asked to rank these drugs according to their personal practice (**Figure 1A**). **Figure 1A** shows that the three most commonly applied non-steroidal immunosuppressants were azathioprine, rituximab and mycophenolate mofetil. Noticeably, mycophenolate mofetil was frequently ranked as second choice, usually after azathioprine. Other immunosuppressants used included sirolimus, cyclosporine, and individual cases of ruxolitinib and tofacitinib.

The majority of respondents had used these drugs only in up to five and occasionally up to 10 patients. Only azathioprine (n=2), MMF (n=1), MTX (n=1) and RTX (n=1) were used in more than 10 patients. Respondents were asked to elaborate on their experience with the immunosuppressants they had ranked first and second. The cumulative responses for the top five non-steroidal agents are shown in **Figure 1B**. Noticeably, although azathioprine was the first choice for most respondents, its perceived effectiveness appeared less favorable than for other drugs; particularly the combination of rituximab with mycophenolate mofetil, but also mycophenolate mofetil alone appeared to induce a response in a larger proportion of the patients. These findings suggest that the choice of drug is not solely based on its expected clinical effectiveness but that other factors are involved; indeed, some respondents mention the costs and availability of rituximab in particular as limiting factors. These answers corresponded with the answers given to the question whether clinicians would discourage the prescription of the particular drug. Mycophenolate mofetil, rituximab and the combination of these two were less likely to be discouraged. Drugs were often discouraged for multiple reasons; usually side effects, but also other effects or ineffectiveness. Hydroxychloroquine was usually discouraged due to a lack of effect.

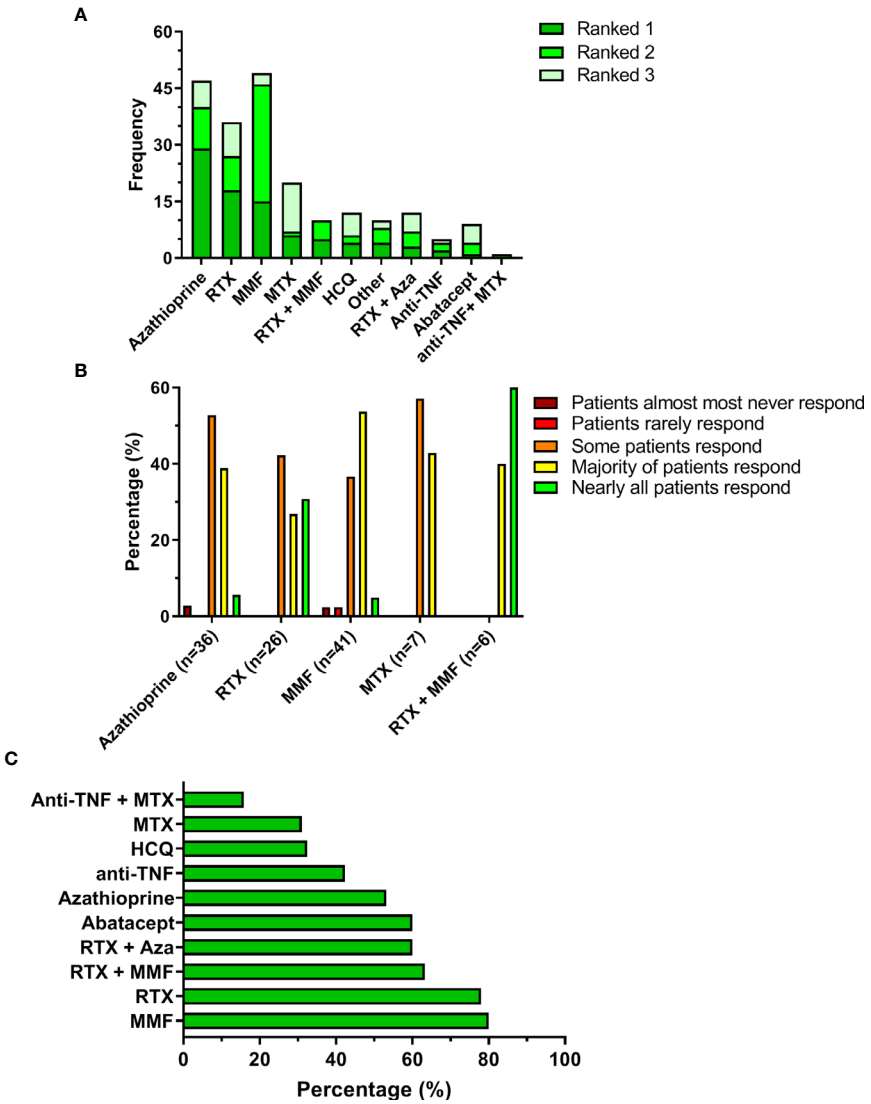

FIGURE 1 | Immunosuppressive therapy in GLILD. **(A)** Top-three ranking of non-steroidal immunosuppressive drugs. **(B)** Estimated patient response rates to the 5 most highly ranked non-steroidal immunosuppressive agents according to the treating clinicians. **(C)** Percentage of respondents that would encourage the use of the non-steroidal immunosuppressive drug. Aza, azathioprine; HCQ, hydroxychloroquine; MMF, mycophenolate mofetil; MTX, methotrexate; RTX, rituximab.

In addition to use of immunosuppressive therapy, respondents were enquired to comment on the use of antimicrobial prophylaxis to prevent *Pneumocystis jiroveci* pneumonia (PCP). Answers were categorized into different categories as shown in **Figure 2**. The prescription of PCP prophylaxis was very heterogeneous, both within and between specialties. PCP prophylaxis appeared to be more frequently applied by adult specialists than by pediatricians, but the differences were not statistically significant. Various comments were given if the option "other" was chosen. PCP prophylaxis was often individualized and based on (combinations of) $CD4^+$ T cell counts, duration of immunosuppressive therapy and combinations of immunosuppressants, particularly the combination of a DMARD with systemic glucocorticoids.

DISCUSSION

We present the results of an online clinician survey related to the diagnosis and management of GLILD. We received 161 responses from physicians caring for GLILD patients all over the world. The results show that there are many areas of need and uncertainty on this topic that deserve attention.

The diagnosis of GLILD is often difficult and most respondents did not have access to a GLILD protocol. CVID disorders patients were often not regularly screened for GLILD using PFT and less frequently by CT. Once GLILD was considered, the diagnosis was usually based on PFT and CT, aided by exclusion of infection *via* auxiliary blood, sputum and

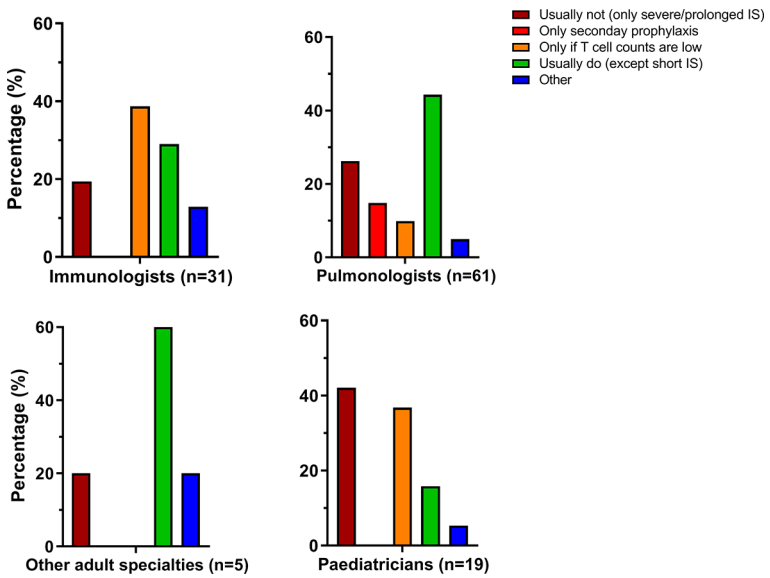

FIGURE 2 | Prescription of *Pneumocytis jiroveci* pneumonia (PCP) antimicrobial prophylaxis varies within and between specialties. IS, immunosuppression.

BAL testing. The necessity of lung biopsy remains controversial. Immunological BAL analysis was not frequently used, likely due to its uncertain value in the diagnosis of GLILD. The majority of respondents experienced difficulty defining adequate follow-up of GLILD patients. Especially, imaging monitoring would benefit from guidelines with considerable heterogeneity in the use and interval of examinations by X-ray and chest CT. Most of these findings are in line with the results of the British Lung Foundation (BLF) survey conducted among UK centers (8), which showed overall consensus regarding the original work-up of GLILD but failed to define consensus related to management strategies and the initiation of therapy in certain patient groups such as asymptomatic GLILD.

Regarding therapy, corticosteroids remain the first line of immunosuppressive induction therapy for the majority of respondents, as is common practice in literature and clinical setting (8, 15). About half of respondents of the BLF survey also use corticosteroids in low dosages to maintain remission. Of those respondents, 46% preferred non-steroidal immunosuppressive monotherapy, 13% corticosteroids alone, 21% a combination of both and 13% complete withdrawal and monitoring. The fact that in our cohort 33% uses a maintenance dose of >7.5 mg/d of prednisone may already hint towards the difficulty of choosing an alternative second line therapy.

This uncertainty is also reflected by the heterogeneous use of non-steroidal immunosuppressive agents which includes cDMARD, biologicals and combinations of both. Within this study, azathioprine, mycophenolate mofetil and rituximab were most frequently used. Indeed, for these three drugs there was 80% or greater consensus with the BLF study. However, although part of the consensus, the frequent use of azathioprine was not based on clinical evidence as a substantial fraction of the respondents did not report azathioprine as being effective in this disease. In contrast, the combination of rituximab with azathioprine first promoted by the early paper of Chase and colleagues (22) has been used successfully in several patients. The successful induction of radiological and spirometric improvement by a combination of rituximab with azathioprine or mycophenolate mofetil was confirmed in a recent extension and expansion of the original Chase study reporting retrospectively 39 GLILD patients with and without an underlying monogenetic defect (28).

In addition to a lack of evidence regarding optimal immunosuppressive therapy, the question of whether PCP prophylaxis should be employed and, if so, in which patients, remains to be answered. Antimicrobial prophylaxis was considered beneficial in a meta-analysis of a heterogeneous population of non-HIV immunocompromised patients (34). As most of these patients had both impaired humoral and cellular immune responses due to acute leukemia or organ transplantation, it remains unclear whether these findings could and should be extrapolated to all GLILD patients. The variable PCP prophylaxis strategies in our survey reflect the lack of recommendations for non-HIV immunocompromised patients. Typically, the decision is made for each case individually, including factors such as combination and duration of immunosuppressive regimen, numbers of CD4+ T lymphocytes and perhaps other elements such as age, comorbidities and physician's preferences.

The strengths of this study include a high response rate of 161 valuable responses from 47 countries, making this the largest survey on this topic until now. Respondents represented six continents and worked in the relevant specialties of pulmonology and immunology for both pediatric and adult patients. These

findings thus provide an adequate reflection of the real practice of managing GLILD in CVID disorders. Detailed responses were provided on multiple relevant subjects, including diagnosis, follow-up and therapy.

Despite our high number of responses, it still represents only a small proportion of the actual population of clinicians that take care of these patients. Hence, certain selection bias cannot be excluded. Additionally, the completeness of the answers is a limitation as it varied from ~65% to 100%. Particularly the section on therapy of GLILD was incomplete and filled out by around two-thirds of the respondents. This can be due to the length of the survey and the fact that treatment is generally carried out in multidisciplinary teams. Additionally, respondents may not feel comfortable regarding their experience with treatment of GLILD, as patient numbers were low and respondents appeared habitually reluctant to initiate therapy and treatment. Finally, this survey shows that a major limitation of current GLILD management is the lack of evidence, for which consensus is a poor substitute. There is a clear need for basic, translational and clinical research in order to eventually establish evidence-based guidelines. Basic research into the pathogenesis of GLILD should aim to elucidate the complex interplay between immune system, local micro-environment of the lungs and microbes (35) and host-microbe interactions. These findings may allow for development of targeted therapies, or optimization of the use of available drugs for improved efficacy and reduced toxicity. Since the clinic-radio-pathological picture of GLILD is very heterogeneous, the pathogenesis is probably multifaceted as well. Therapy should be optimized on the specific subtype of GLILD, perhaps eventually guided by the cellular infiltrates on biopsy, while taking into account other relevant factors such as toxicity, availability and patient preferences. Despite the pressure to see patients virtually in the current COVID-19 pandemic, this population requires face-to-face contact including clinical and diagnostic exams.

The rarity of GLILD remains an Achilles' heel, as further dissection of this relatively small cohort into more homogenous subgroups relies on international collaboration between GLILD clinicians. Collaborative clinical studies addressing natural disease course, prognosis and treatment outcomes ought to be performed in multicenter, standardized settings. The development of an expert platform to collect data should be encouraged, as well as biobanking of biopsy specimens. Awareness, education and the availability of facilities for low-income countries are important additional topics.

The European Respiratory Society recognizes these needs and supported the launch of a Clinical Research Collaboration on GLILD, the e-GLILDnet (https://www.ersnet.org/research/e-glildnet—a-european-granulomatous-lymphocytic-interstitial-lung-disease-network; twitter: @glildnet). The e-GLILDnet aims to bring together clinicians, researchers and patients representatives from across Europe to improve the lives of those living with GLILD.

In conclusion, our survey data demonstrate an urgent need for clinical studies to provide more evidence for an international consensus regarding diagnosis and management of GLILD. The e-GLILDnet will support and facilitate this aim by supporting international collaboration, particularly on studies addressing optimal procedures for definite diagnosis and a better understanding of the pathogenesis of GLILD in order to provide individualized treatment options.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors upon request, without undue reservation.

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

All authors were responsible for drafting the survey. AR finalized the survey and gathered the raw data. TA and AV performed the data analyses. AV drafted the figures. AV, TA, KW, and JH drafted the paper outline. AV, TA, and AR wrote the paper, supervised by KW and JH. All authors contributed to the article and approved the submitted version.

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SYSTEMATIC REVIEW
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What Works When Treating Granulomatous Disease in Genetically Undefined CVID? A Systematic Review

Astrid C. van Stigt[1,2,3], Willem A. Dik[1,3], Lieke S. J. Kamphuis[3,4], Bas M. Smits[5], Joris M. van Montfrans[5], P. Martin van Hagen[1,2,3], Virgil A. S. H. Dalm[1,2,3†] and Hanna IJspeert[1,3†]*

[1] Laboratory Medical Immunology, Department of Immunology, Erasmus University Medical Center, Rotterdam, Netherlands, [2] Department of Internal Medicine, Division of Clinical Immunology, Erasmus University Medical Center, Rotterdam, Netherlands, [3] Academic Center for Rare Immunological Diseases (RIDC), Erasmus University Medical Center, Rotterdam, Netherlands, [4] Department of Pulmonary Medicine, Erasmus University Medical Centre, Rotterdam, Netherlands, [5] Department of Pediatric Immunology and Rheumatology, Wilhelmina Children's Hospital, University Medical Centre (UMC), Utrecht, Netherlands

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***Correspondence:**
Hanna IJspeert
h.ijspeert@erasmusmc.nl

[†]These authors have contributed equally to this work

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Background: Granulomatous disease is reported in at least 8–20% of patients with common variable immunodeficiency (CVID). Granulomatous disease mainly affects the lungs, and is associated with significantly higher morbidity and mortality. In half of patients with granulomatous disease, extrapulmonary manifestations are found, affecting e.g. skin, liver, and lymph nodes. In literature various therapies have been reported, with varying effects on remission of granulomas and related clinical symptoms. However, consensus recommendations for optimal management of extrapulmonary granulomatous disease are lacking.

Objective: To present a literature overview of the efficacy of currently described therapies for extrapulmonary granulomatous disease in CVID (CVID+EGD), compared to known treatment regimens for pulmonary granulomatous disease in CVID (CVID+PGD).

Methods: The following databases were searched: Embase, Medline (Ovid), Web-of-Science Core Collection, Cochrane Central, and Google Scholar. Inclusion criteria were 1) CVID patients with granulomatous disease, 2) treatment for granulomatous disease reported, and 3) outcome of treatment reported. Patient characteristics, localization of granuloma, treatment, and association with remission of granulomatous disease were extracted from articles.

Results: We identified 64 articles presenting 95 CVID patients with granulomatous disease, wherein 117 different treatment courses were described. Steroid monotherapy was most frequently described in CVID+EGD (21 out of 53 treatment courses) and resulted in remission in 85.7% of cases. In CVID+PGD steroid monotherapy was described in 15 out of 64 treatment courses, and was associated with remission in 66.7% of cases. Infliximab was reported in CVID+EGD in six out of 53 treatment courses and was mostly used in

granulomatous disease affecting the skin (four out of six cases). All patients (n = 9) treated with anti-TNF-α therapies (infliximab and etanercept) showed remission of extrapulmonary granulomatous disease. Rituximab with or without azathioprine was rarely used for CVID +EGD, but frequently used in CVID+PGD where it was associated with remission of granulomatous disease in 94.4% (17 of 18 treatment courses).

Conclusion: Although the number of CVID+EGD patients was limited, data indicate that steroid monotherapy often results in remission, and that anti-TNF-α treatment is effective for granulomatous disease affecting the skin. Also, rituximab with or without azathioprine was mainly described in CVID+PGD, and only in few cases of CVID+EGD.

Keywords: common variable immune deficiency, granulomatous disease, lung, immunosuppressive therapy, extrapulmonary

INTRODUCTION

Common variable immunodeficiency (CVID) is a primary antibody deficiency with a heterogeneous clinical phenotype. It is characterized by a marked decrease in levels of immunoglobulin (Ig) G with decreased levels of IgA and/or IgM, and an impaired response to immunization (1, 2). Recurrent infections, mainly by encapsulated bacteria, are a clinical hallmark in the majority of CVID patients. Furthermore, large cohort studies showed that up to 74% of CVID patients suffer from non-infectious complications (3, 4). These include granulomatous disease, progressive lung disease, autoimmunity (AI), enteropathy, liver disease, and malignancy (3, 4). These non-infectious complications are associated with deleterious effects on disease burden and survival, as the presence of one or more of these non-infectious complications results in ~11 times higher risk of death compared to CVID patients with infectious complications only (5).

Granulomatous disease is reported in 8–20% of CVID patients (3, 4, 6), although it is generally assumed that the presence of granulomatous disease is underreported. The trigger for granuloma formation in CVID remains elusive. The long-standing observation of an increased incidence of autoimmune disease in CVID patients with granulomatous disease could suggest an immune dysregulated milieu that supports granuloma formation (7, 8). Various infectious triggers have been reported as well. Human Herpes virus-8 and *Toxoplasma gondii* are reported in relation to granuloma formation in CVID (9, 10). More recently, Rubella positive M2 macrophages were identified in granulomas in a patient with CVID that received a Rubella vaccine during childhood (11). However, reports are limited or could not be reproduced and further research is required to better understand the pathogenesis of granulomatous disease in CVID. In CVID patients, granulomatous disease mainly affects the lungs, followed by lymph nodes (LN) and liver (3, 8). Granulomatous disease of the lungs can be accompanied by interstitial lymphocytic infiltrates, referred to as granulomatous lymphocytic interstitial lung disease (GLILD), a condition not exclusively observed in CVID. The lungs as site for complications in primary antibody deficiencies, both infectious or non-infectious related, is extensively discussed in the paper by Bauman et al. (12). They highlight the heterogeneity in diagnostic procedures and lack of guidelines for the treatment of non-infectious complications, including GLILD, in primary antibody deficiencies such as CVID. GLILD is a severe complication, as shown by Bates et al. as they observed GLILD in CVID to be associated with a 50% reduction of survival probability when compared to CVID patients without this complication (13). Over the past years, there has been much focus on the diagnostic process and treatment of granulomatous disease affecting the lungs (14). However, extrapulmonary granulomatous disease is reported in about half of the patients with granulomatous disease, making this subgroup at least as important (3). Granulomatous lesions are reported in the LN, liver, spleen, gastrointestinal tract (GI tract), bone marrow (BM), skin, eyes, central nervous system (CNS), parotid gland, and kidneys (7, 15–20). Interestingly, patients with extrapulmonary granulomatous disease have a higher incidence of autoimmune diseases compared to patients with granuloma restricted to the lungs (7, 15).

Immunoglobulin replacement therapy (IgRT) is one of the cornerstones of therapy in CVID, and has reduced the risk of severe infectious complications (21). A protective effect of IgRT on development of autoimmune disease, including autoimmune hemolytic anemia (AIHA) and immune thrombocytopenia (ITP), has been proposed (22). Optimizing treatment of granulomatous disease is amongst the major challenges in current clinical practice for CVID patients. Various therapies for granulomatous disease, varying from classical immunosuppressive agents, including steroids, and disease modifying anti-rheumatic drugs (DMARDs), to more specific biologics such as rituximab, have been reported; each with varying effects on remission of granulomatous lesions and clinical improvement (23). Moreover,

Abbreviations: AIHA, Autoimmune hemolytic anemia; BM, Bone marrow; CNS, Central nervous system; CVID, Common variable immune deficiency; EGD, Extrapulmonary granulomatous disease; GI, Gastro intestinal; GLILD, Granulomatous lymphocytic interstitial lung disease; HSCT, Hematopoietic stem cell transplantation; IFN, Interferon; IgRt, Immunoglobulin replacement therapy; ITP, Immune thrombocytopenic purpura; LN, Lymph node; MMF, Mycophenolate mofetil; MTX, Methotrexate; PGD, Pulmonary granulomatous disease; TNF, Tumor necrosis factor.

there is a diversity of combinations of immunosuppressive treatments, resulting in a diverse group of multi-drug treatment regimens.

Over the past decades, many reports have been published containing valuable information regarding treatment of granulomatous disease in CVID. With this systematic review, we aim to provide an overview of the currently described treatment regimens for granulomatous disease in genetically undefined CVID with a special focus on treatment for extrapulmonary granulomatous manifestations, and to report which of these treatments are associated with remission of granulomatous disease. We compared treatment regimens for extrapulmonary granulomatous disease with regimens used in granulomatous disease with lung involvement. Taking these efforts together, we aim to elucidate which treatment regimens are associated with remission of extrapulmonary granulomatous disease.

METHODS

Search Strategy and Article Identification

We performed a systematic search to identify all manuscripts that describe the effect of drug therapy on clinical outcome of granulomatous disease in CVID patients. The following databases were used: Embase, Medline(Ovid), Web-of-Science Core Collection, Cochrane Central, and Google Scholar, using specific search strings per database (**Table 1**, **Figure 1**). Only English-language peer-reviewed articles were included, conference abstracts were excluded. On December 5th 2019,

TABLE 1 | Overview of databases and search strings.

Database	Search string
Embase.com	('granuloma'/exp OR (granulom*):ab,ti,kw) AND ('common variable immunodeficiency'/de OR (CVID* OR ((variable*) NEAR/3 (immunodefi* OR agammaglobulinaem* OR hypogammaglobulinaem* OR hypogammaglobulinem* OR immune*-deficien*))):ab,ti,kw) NOT ([Conference Abstract]/lim) AND [ENGLISH]/lim
Medline(Ovid)	(exp "Granuloma"/OR (granulom*).ab,ti,kw.) AND ("Common Variable Immunodeficiency"/OR (CVID* OR ((variable*) ADJ3 (immunodefi* OR agammaglobulinaem* OR hypogammaglobulinaem* OR hypogammaglobulinem* OR immune*-deficien*))).ab,ti,kw.) NOT (news OR congres* OR abstract* OR book* OR chapter* OR dissertation abstract*).pt. AND (english).lg
Web-of-Science Core Collection	TS=(((granulom*)) AND ((CVID* OR ((variable*) NEAR/2 (immunodefi* OR agammaglobulinaem* OR hypogammaglobulinaem* OR hypogammaglobulinem* OR immune*-deficien*))))) AND DT=(Article OR Review) AND LA=(English)
Cochrane Central	((granulom*):ab,ti,kw) AND ((CVID* OR ((variable*) NEAR/3 (immunodefi* OR agammaglobulinaem* OR hypogammaglobulinaem* OR hypogammaglobulinem* OR immune* NEXT deficien*))):ab,ti,kw)
Google Scholar	Granuloma "Common Variable Immunodeficiency"|CVID lung|pulomonary

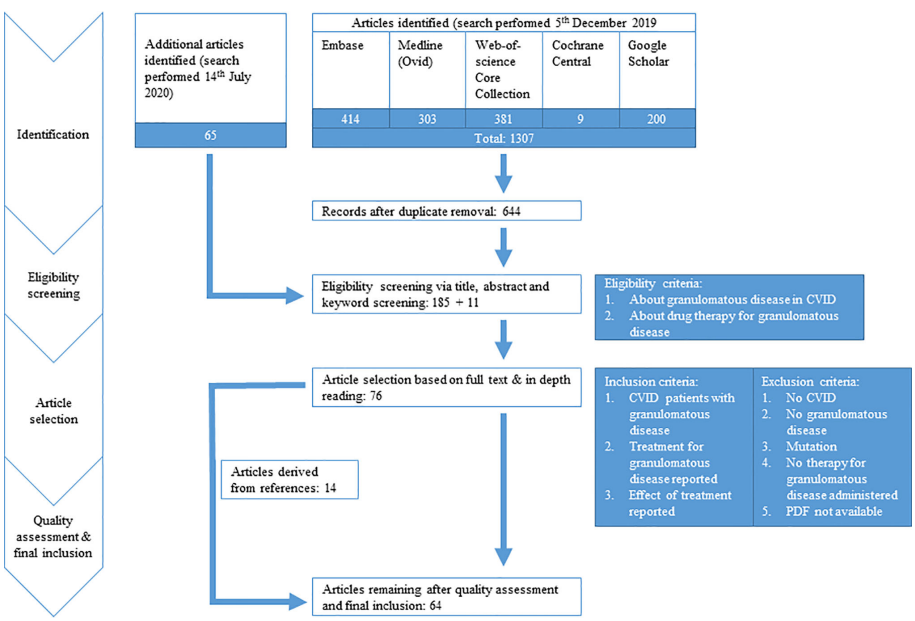

FIGURE 1 | PRISMA flow diagram. PRISMA diagram showing search strategy and inclusion process of articles. Common variable immune deficiency (CVID).

after correcting for duplicate findings, a total of 644 articles was obtained for initial screening for eligibility (**Table 2**). An update on the performed systematic search was performed July 14th 2020, obtaining 65 articles.

Eligibility Screening

Of these 709 (644 + 65) articles, title and abstract were screened for eligibility by two independent reviewers (HIJ and AS), with a third reviewer (VD) being involved when a discrepancy existed between the two primary reviewers. Articles were considered to be eligible when the title and/or abstract and/or keywords referred to the effect of drug therapy on granulomatous disease in CVID patients. In case the abstract, title, or keywords did not suggest that the manuscript focused on CVID, granulomatous disease, drug therapy, and effect on clinical outcome, the article was excluded. For articles where no abstract was available, such as letters, full text articles were screened for eligibility. Hereby, 196 (185 + 11) articles were selected.

Article Selection, Quality Assessment, and Final Inclusion

The selected 196 articles were used for full in-depth reading by the two independent reviewers (HIJ, AS). Articles were included when the following inclusion criteria were met: 1) CVID patients with granulomatous disease, objectified prior to treatment by clinician *via* biopsy/radiographic imaging/functional analysis (pulmonary function testing, ocular examination)/clinical assessment, 2) treatment for granulomatous disease reported, and 3) outcome of treatment evaluated *via* radiographic imaging/functional testing/clinical assessment. Exclusion criteria were: 1) papers not describing CVID, 2) not about granulomatous disease, 3) patients with genetic defects reported, 4) no therapy administered for granulomatous disease, or 5) PDF not obtainable (**Figure 1**). Articles describing sarcoidosis in CVID patients, or describing CVID patients with sarcoidosis-like granulomatous disease, were included in the analysis. Hereby, 76 articles were included. Next, quality assessment was performed. For included case-control studies (n = 1), the Newcastle-Ottawa Quality Assessment Scale for Case Control Studies was used (http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp, **Supplemental Table 1**). For case reports and case series (n = 75), the assessment tool described by Murad et al. was used (https://ebm.bmj.com/content/23/2/60 **Supplemental Table 2**) (24). Articles with a poor quality score (≤2) were excluded for data extraction. By cross-reference checking, 14 additional articles were identified. After eligibility screening and in-depth reading, three of these 14 manuscripts were of sufficient quality and included. Hereby, 64 articles were finally included in this systematic review and used for data extraction and analysis (**Figure 1**, **Supplemental Table 3**).

Data Extraction and Data Analysis

Of the 64 articles finally included, reported study characteristics and outcome measures were collected and summarized (**Supplemental Table 4**). A total of 95 CVID cases with granulomatous disease were used for further analysis. Since we aimed to examine whether there was a difference regarding treatment and treatment efficacy between CVID patients with extrapulmonary granulomatous disease (CVID+EGD) and CVID patients with pulmonary granulomatous disease (CVID +PGD), patients were categorized based on granuloma locations reported: CVID+EGD for patients with exclusively extrapulmonary granuloma, and CVID+PGD for patient with pulmonary granuloma (with or without granuloma at other sites).

Treatment regimens and effect on granulomatous disease were extracted for each case. In various reported cases, multiple treatment regimens were administered. When multiple treatment regimens were applied for granulomatous disease within one patient at different time points, the effect of the treatment regimens was considered separately. The efficacy of a specific treatment regimen, i.e. the association with remission of granulomatous disease, was evaluated per treatment course of this treatment regimen. The efficacy of treatment regimens was determined based on either one or more of the following reported findings described in the included articles: 1) reported improvement in clinical presentation, 2) reported improvement of radiological findings, 3) reported improvement of specific function testing, such as lung function testing (for lung granulomatous disease) or ocular examination (for granulomatous disease affecting the eye). Per treatment regimen, the number of courses reported and the number of courses associated with remission were scored. In some cases, administration of IgRT as additional therapy was reported. When IgRT was initiated simultaneously with therapy for granulomatous disease, IgRT was considered part of the treatment regimen for granulomatous disease, as it could not be ruled out that IgRT had an effect on granulomatous disease. When IgRT was started before the treatment regimen aimed at granulomatous disease, IgRT was not considered as treatment of granulomatous disease.

TABLE 2 | Overview of database and output of search.

Database	Number of references	Number of references after duplication
Embase.com	414	407
Medline(Ovid)	303	31
Web-of-Science Core Collection	381	131
Cochrane Central	9	6
Google Scholar	200	69
Total 5th of December 2020	1,307	644
Total updated search 14th of July 2020		65
Final total references screened		**709**

RESULTS

Study Selection and Literature Cases Characteristics

After searching databases, 709 articles were screened for eligibility. Full text reading and quality assessment resulted in 64 articles for data extraction (**Figure 1**, **Supplemental Tables 1–4**). From the 64 articles, a literature derived cohort of 95 patients was obtained (**Table 3**). The cases were divided in two groups: 1) CVID patients with extrapulmonary granulomatous disease only (CVID+EGD; n = 44; 46.3%) and 2) CVID patients with pulmonary granulomatous disease (CVID+PGD; N = 51; 53.7%) (**Table 3**). The overall ratio female/male was 2.2 (female n = 65; male n = 30), with a slightly higher ratio in the CVID+PGD group *versus* the CVID+EGD group (2.6 *vs* 1.8, respectively). The average age, based on age reported in article or age when CVID was diagnosed, was 34.3 with a range 2–72 years. In 83.2% (79 out of 95) of the patients, biopsy was obtained as part of the diagnostic work-up for granulomatous CVID. In the remaining 16 cases, clinical assessment, ocular examination, (HR)CT or MRI were used to diagnose granulomatous disease. In 63.2% of all cases (60 of 95), we were able to determine whether granulomatous disease was present before or after CVID was diagnosed. In 36.7% (22 of 60) of the patients, granulomatous disease was diagnosed before the diagnosis of CVID. In the CVID+EGD group in 30.0% of patients (9 out of 30 patients) granulomatous disease was diagnosed before diagnosis of CVID, while in the CVID+PGD group this was 43.3% (13 out of 30 patients). Within this literature derived cohort the lungs, skin, LN, liver, eye, spleen, intestines, kidneys, conjunctiva, CNS, and vocal cords were affected by granulomatous disease (**Table 4**, **Supplemental Table 5**). Of note, within one patient multiple organs could be involved (**Supplemental Table 5**). Overall, pulmonary granulomatous disease was the most frequently affected location (n = 51), followed by skin (n = 24) and LN (n = 20) (**Table 4**).

Administered Treatment Regimens in Granulomatous Disease in CVID

Steroids

Steroid therapy was the most frequently reported treatment regimen for granulomatous disease in CVID (**Tables 5** and **6**). For CVID+EGD, steroid monotherapy was the most frequently reported regimen (21 of 53 treatment courses), with 85.7% of treatment courses scored as effective (**Table 5**) (17, 19, 20, 25–40). For CVID+PGD, steroid monotherapy also was the most frequently reported treatment regimen (15 of 64 treatment courses); 66.7% of these treatment courses were associated with remission of granulomatous disease (**Table 6**) (29, 41–50). Apart from monotherapy, steroids were frequently prescribed as part of a treatment regimen containing one or more other drugs, both in CVID+EGD and CVID+PGD. However, the duration, type, and doses administered varied between the different studies. Overall, these results suggest that steroid therapy is a beneficial therapeutic option, either as monotherapy or as part of combination therapy, for granulomatous disease in CVID.

TABLE 4 | Reported granuloma involvement per organ location.

Organ location	Number reported
lung	51
skin	24
LN	20
liver	16
eye	12
spleen	6
intestinal	5
kidney	3
conjunctiva	2
CNS	1
vocal cords	1
total	**141**

Multiple organs can be affected per patient; thus, in 95 patients, 141 granuloma locations were scored.

TABLE 3 | Characteristics of 96 literature cases derived from 64 articles.

Characteristics included literature cases	Total cases	Extra pulmonary granulomatous disease cases (CVID+EGD)	Pulmonary granulomatous disease cases (CVID+PGD)
Number of patients	95 (100%)	44 (46.3%)	51 (53.7%)
Ratio female/male	2.2 (65/30)	1.8 (28/16)	2.6 (37/14)
Age of diagnosis CVID or age reported in article:	34.3	35.0	33.7
• Average			
• Min. of age	2	4	2
• Max. of age	72	72	68
Biopsy obtained for diagnosis granuloma (% of total number within group)	79 (83.2% of 95)	37 (84.1% of 44)	42 (82.4% of 51)
Timing diagnosis granuloma *vs* diagnosis CVID known	60 (63.2% of 95)	30 (68.2% of 44)	30 (58.8% of 51)
Granuloma diagnosed before diagnosis CVID	22 (36.7%)	9 (30.0%)	13 (43.3%)
Granuloma diagnosed after diagnosis CVID	38 (63.3%)	21 (70.0%)	17 (56.6%)
Timing diagnosis granuloma *vs* diagnosis CVID not known, or same time point	35 (36.8% of 95)	14 (31.8%)	21 (41.2%)
Number of treatment courses administered for granulomatous disease	117 (100%)	53 (45.3%)	64 (54.7%)

Characteristics of literature derived cohort. Percentages are of relevant totals shown.

However, various studies reported relapse of granulomatous disease after discontinuation or termination of steroid therapy, in both the CVID+EGD (17, 30, 34, 39, 51, 52) and CVID+PGD (39, 42, 47, 48, 53, 54) group.

Infliximab and Etanercept

In CVID+EGD cases, the TNF-α inhibitor infliximab was the third most frequently reported treatment regimen (six out of 53 treatment courses) (**Table 5**). Infliximab as monotherapy was always associated with remission (**Table 5**) (28, 30, 33, 55). In four out of six patients, infliximab was used to treat granulomatous disease of the skin (28, 30, 33, 55). One study reported a treatment regimen of steroids with infliximab for granulomatous disease of the eye, which did not result in remission of granulomatous disease (37). In CVID+PGD, infliximab was less frequently reported as monotherapy (two out of 64 treatment courses), and in one patient infliximab was administered in combination with IgRT (**Table 6**) (54, 55). These three treatment courses were associated with remission in the CVID+PGD group.

Etanercept, also interfering in the TNF-α signaling cascade, was described only in CVID+EGD (three out of 53 treatment courses) (**Table 5**). All three cases suffered from granulomatous disease of the skin without other organ involvement (18, 56, 57). All treatment courses with etanercept were associated with remission in CVID+EGD.

Rituximab With or Without Azathioprine

Both rituximab and azathioprine were rarely administered in the CVID+EGD group (**Table 5**). Only two cases with either rituximab or azathioprine were described. One study reported rituximab in combination with steroids in the CVID+EGD group, which was associated with remission of extrapulmonary granulomatous disease of the kidney (**Tables 4** and **5**) (58). Another study reported a patient with granulomatous disease of the skin, where steroids with azathioprine were administered; this was associated with remission of granulomatous disease (**Tables 4** and **5**) (52). Within the CVID+PGD group, the combination of rituximab with azathioprine was the second most frequently reported treatment regimen (12 out of 64 treatment courses), and was associated with remission in 11 of the 12 treatment courses (91.7%) (**Table 6**) (49, 59–62). Also, two treatment courses in the CVID+PGD were reported where steroids formed part of the treatment regimen together with rituximab and azathioprine (63, 64), and one where azathioprine was given with steroids (65). All of these treatment courses were considered effective as treatment for granulomatous disease. Rituximab as monotherapy was the third most frequently reported treatment regimen in CVID+PGD (six out of all 64 treatment courses), and the third most frequent treatment regimen associated with remission (six out of 51 treatment courses associated with remission) (**Table 6**) (66–68). All described treatment courses of rituximab monotherapy for CVID+PGD were effective (**Table 6**). In 20 of the 22 patients with CVID+PGD were rituximab was part of treatment regimen, granulomatous disease was only present in the lungs (**Supplemental Table 4**) (49, 59–64, 66–69). In the majority of the included cases the dose of rituximab as part of combination therapy with azathioprine was consistent, namely 375 mg/m2 (49, 59, 60, 62). However, the duration of therapy when retrievable varied greatly, from one time administration to 4 weeks or 6 months of treatment.

Immunoglobulin Replacement Therapy

We observed IgRT monotherapy to be the second most frequently prescribed treatment regimen for CVID+EGD (six out of 53 treatment courses). Three out of the six treatment courses were associated with remission (**Table 5**) (39, 70–73). In the CVID+PGD group, IgRT monotherapy was also reported, of

TABLE 5 | Treatment regimen and number of treatment courses administered in CVID+EGD group.

Treatment regimens in CVID+EGD	Total	Treatment courses with remission	Treatment courses without remission
steroids	21	18 (85.7%)	3 (14.3%)
IgRT	6	3 (50%)	3 (50%)
infliximab	6	6 (100%)	0 (0%)
steroids with IgRT	4	4 (100%)	0 (0%)
etanercept	3	3 (100%)	0 (0%)
anti-mycobacterial therapy	1	0 (0%)	1 (100%)
adalimumab	1	0 (0%)	1 (100%)
antibiotics with steroids	1	0 (0%)	1 (100%)
antibiotics, anti-fungal therapy, steroids, cyclosporine, hydroxychloroquine, IFN-γ, MTX	1	0 (0%)	1 (100%)
cyclophosphamide	1	1 (100%)	0 (0%)
cyclosporine	1	1 (100%)	0 (0%)
IFN-alpha with anti-mycobacterial therapy	1	1 (100%)	0 (0%)
MMF	1	1 (100%)	0 (0%)
steroids with anti-mycobacterial therapy	1	0 (0%)	1 (100%)
steroids with azathioprine	1	1 (100%)	0 (0%)
steroids with infliximab	1	0 (0%)	1 (100%)
steroids with methotrexate	1	1 (100%)	0 (0%)
steroids with rituximab	1	1 (100%)	0 (0%)
Total	**53**	**41**	**12**

IgRT, immunoglobulin replacement therapy; IFN, interferon; MTX, methotrexate; MMF, mycophenolate mofetil. Percentages are of total number of treatment courses per treatment regimen.

TABLE 6 | Treatment regimen and number of treatment courses administered in CVID+PGD group.

Treatment regimen in CVID+PGD	Total	Treatment courses with remission	Treatment courses without remission
steroids	15	10 (66.7%)	5 (33.3%)
rituximab with azathioprine	12	11 (91.7%)	1 (8.3%)
rituximab	6	6 (100%)	0 (0%)
steroids with IgRT	6	4 (66.7%)	2 (33.3%)
IgRT	5	4 (80%)	1 (20%)
MMF	3	3 (100%)	0 (0%)
anti-mycobacterial therapy	2	0 (0%)	2 (100%)
IgRT with MMF	2	2 (100%)	0 (0%)
infliximab	2	2 (100%)	0 (0%)
steroids with rituximab with azathioprine	2	2 (100%)	0 (0%)
cyclophosphamide	1	0 (0%)	1 (100%)
IgRT with infliximab	1	1 (100%)	0 (0%)
IgRT with methotrexate with hydroxychloroquine	1	1 (100%)	0 (0%)
IgRT with rituximab	1	1 (100%)	0 (0%)
rituximab with MMF	1	1 (100%)	0 (0%)
steroids with azathioprine	1	1 (100%)	0 (0%)
steroids with cyclophosphamide	1	1 (100%)	0 (0%)
steroids with cyclosporine	1	1 (100%)	0 (0%)
steroids with IgRT with anti-mycobacterial therapy	1		1 (100%)
	64	**51**	**13**

IgRT, immunoglobulin replacement therapy; IFN, interferon; MMF, mycophenolate mofetil. Percentages are of total number of treatment courses per treatment regimen.

which four of the total five treatment courses were associated with remission of granulomatous disease (**Table 6**) (64, 74–77). The treatment regimen consisting of IgRT with steroids was reported four times in CVID+EGD; all were associated with remission of granulomatous disease (**Table 5**) (36, 39, 51). Within the CVID+PGD group, steroids with IgRT was used in six out of all 64 treatment courses, of which four were associated with remission of pulmonary granulomatous disease (**Table 6**) (36, 39, 50, 54, 78).

Other Treatment Regimen

The remaining therapeutic regimens reported in the included articles were diverse, and low in frequency; most of these treatment regimen had only one treatment course (**Tables 5** and **6**, **Supplemental Table 4**) (19, 33, 38, 39, 41, 48, 50, 52–54, 56, 57, 64, 78–83). Cyclophosphamide, cyclosporine, hydroxychloroquine, methotrexate, mycophenolate mofetil, among others were reported in our literature derived cases. They were mainly administered in combination with other immunosuppressive medication and generally associated with a remission of granulomatous disease, for both CVID+PGD as well as CVID+EGD.

DISCUSSION AND CONCLUSION

Randomized controlled clinical trials for the treatment of granulomatous disease in CVID are lacking. Currently, attention for treatment of granulomatous disease in CVID has mostly focused on GLILD (14). In 2017 the British lung foundation and United Kingdom primary immunodeficiency network published a consensus statement for the management of GLILD in CVID based on the experience of 33 consultants from the United Kingdom (14). It was proposed to use oral steroids as first-line treatment, and azathioprine, rituximab, and mycophenolate alone or in combination with steroids as second-line treatment. In this systematic review we summarized current literature on the treatment of extrapulmonary granulomatous disease and compared it to the treatment of pulmonary granulomatous disease. We included CVID patients with granulomatous disease in the lungs and excluded CVID patients that had interstitial lung disease without granuloma. Also, patients with known genetic variants were excluded, since potential pathogenic pathways could be determined and specific targeted therapies could be considered.

In about half of the CVID patients with granulomatous disease, extrapulmonary involvement is found (3). Moreover, besides lung granulomas, granulomas in the liver are associated with reduced survival (3, 5). Within our literature derived cohort, liver involvement was the fourth most frequently reported organ involved in granulomatous disease. It is interesting to see that the lungs and skin, two organs greatly exposed to the external milieu, form the majority of organs affected by granulomatous disease in the literature derived cases. Additionally, both in the CVID +PGD and CVID+EGD cases, lymph nodes were the second most frequently reported affected organs. This is similar to previous other studies where anatomical locations of granulomatous disease in larger patient series are reported (3, 8).

More than half of the 44 patients with CVID+EGD received steroids as monotherapy or in combination with other therapies. This is in line with the consensus statement on treatment of GLILD by Hurst et al. (14). In the majority of patients, treatment regimens with steroids appeared effective for treatment of granulomatous disease. Also for the CVID+PGD group, treatment regimens containing steroids were frequently associated with remission of granulomatous disease. Lamers et al. summarized the current literature on the treatment of GLILD in CVID (Lamers et al., manuscript submitted). They showed that steroids failed to induce remission in 57% of the patients. This seems less effective than we have reported in this

systematic review. One important difference is that we used a different search strategy and inclusion criteria. Secondly, Lamers et al. included all CVID patients with GLILD, while we did not include CVID patients that had interstitial lung disease without granulomatous disease. Thirdly, we reported treatment as effective when a treatment course was associated with remission regardless whether the granulomatous disease relapsed after termination of treatment. Lamers et al. considered treatment effective only when there was relapse free improvement of the granulomatous disease. These differences in approach could explain the difference regarding efficacy of steroid therapy for granulomatous disease with lung involvement between the two reviews. Both studies observed that discontinuation of steroid therapy could result in recurrence of granulomatous disease. As reported in seven case reports where steroids were administered as monotherapy, initial association with remission of granulomatous disease was observed, but not maintained after discontinuation of steroid therpy (42). (17, 34, 47, 48, 51) These relapses after discontinuation of steroid therapy suggest steroid monotherapy not to have an sustained effect on granulomatous disease. This indicates a potential need for long term therapy, or combination therapy with other immunosuppressive therapy, to maintain granulomatous remission. However, multiple side effects of steroid therapy, together with the dilemma of administering long term immunosuppressive therapy to an immune deficient patient, underscore the need for more targeted, preferably temporarily, therapeutic options.

Granulomatous disease is thought to be initiated, as yet by an unknown trigger, by $CD4^+$ T lymphocytes that, while interacting with antigen presenting cells, become activated (84). Activated $CD4^+$ T lymphocytes secrete cytokines that subsequently stimulate macrophage activation and TNF-α production, ultimately leading to the characteristic immune cell agglomerates (i.e. granulomas) in the involved organs. Like infliximab, etanercept functions by interfering in the TNF-α signaling cascade. Therefore, TNF-α is a theoretically promising cytokine to inhibit in the context of granulomatous disease. Another encouraging finding is the observed improvement of lung function in patients suffering from pulmonary sarcoidosis after treatment with infliximab. However, multiple adverse events are reported for infliximab and etanercept when prescribed for other immune-mediated diseases, such as increased risk of (granulomatous) infections, especially tuberculosis infections, malignancies, and dermatological complications (85–87). Moreover, several cases are reported where TNF-alpha antagonist therapy seemed associated with sarcoid-like disease (88–91).Therefore, TNF-alpha inhibition, although a logical choice for granulomatous disease, should be considered with caution. Within the CVID+EGD patients, infliximab and etanercept were the most frequently used targeted therapies. Moreover, all the infliximab or etanercept based treatment regimens were associated with remission of extrapulmonary granulomatous disease, though the total number of treatment courses with etanercept was limited. In the majority of these cases, granulomatous disease was manifested in the skin (18, 28, 30, 33, 55–57). A beneficial effect of TNF-α inhibition on granulomatous skin disease is also observed in patients suffering from sarcoidosis (92–94). An illustrative case series by Tuchinda et al., presented three patients that received infliximab for sarcoidosis of the skin showing substantial improvement, of which one showed improvement on infliximab monotherapy. Interestingly, all these patients had received previous treatment with immunosuppressive medication, such as steroids, hydroxychloroquine or methotrexate, without clear improvement of lesions (92). The hypothesis of inhibiting granuloma formation by inhibiting the effect of TNF-α either *via* infliximab or etanercept, together with the observed relatively high association with granuloma remission of this treatment regimen, is promising for extrapulmonary granulomatous disease in CVID, especially concerning granulomatous disease of the skin.

Other targeted treatment regimens that were reported, included rituximab and azathioprine. Rituximab is a monoclonal antibody targeting CD20 on B lymphocytes; binding to the Fc-domain eventually results in apoptosis of B-lymphocytes. Rituximab is used in various immune mediated or malignant diseases, and is frequently prescribed in combination with azathioprine, a purine-antagonist of DNA synthesis supposed to halt B- and T-lymphocyte proliferation (95, 96). Of note, within the context of other inflammatory diseases such as rheumatoid arthritis and irritable bowel syndrome, adverse events are reported for rituximab and azathioprine, such as increased risk for infections or malignancies due to their immunosuppressive effects (97, 98). Also certain late adverse events of rituximab, although rare, are reported (99). In CVID, the administration of rituximab has been used effectively for non-infectious complications such as ITP or AIHA (100), and also for GLILD (96, 101). The therapeutic combination of rituximab with azathioprine, is also reported to be beneficial for GLILD (49, 96). The use of rituximab or azathioprine, together with steroids and both effective, was only reported in two patients in the CVID+EGD patients. This is in contrast to what we observed in the CVID+PGD patients, where a treatment regimen of rituximab with azathioprine was the second most frequently reported treatment regimen, and most frequently associated with remission of granulomatous disease. The observed beneficial effect of rituximab and azathioprine for pulmonary granulomatous disease is in line with recent reports on the treatment of GLILD (14, 96). Importantly, the recent paper by Verbsky et al., not included in our analysis because of publication date, showed that rituximab-containing therapeutic regimens improved pulmonary function and radiographic abnormalities in CVID patients with GLILD (96). Rituximab and azathioprine, with the addition of steroids, could be beneficial in CVID+EGD cases, since both included studies reported remission of disease in CVID+EGD patients (52, 58). Due to the limited number of patients treated with rituximab and/or azathioprine CVID+EGD, their effects remain to be elucidated in CVID+EGD.

We found several reports with IgRT as, or as part of, therapy for granulomatous disease (36, 39, 50, 51, 54, 64, 70–78, 102). Since IgRT is the corner stone of treatment in CVID, this treatment regimen is the hardest to judge for being associated with remission of granulomatous disease. The reason for this is twofold. Firstly, as this mode of therapy is considered standard of care, IgRT was not always specifically reported in the included

articles, and can therefore be missed as part of treatment regimens with other therapeutic interventions in our literature cohort. On the other hand, not every CVID patient has a need for IgRT, making the absence of reported IgRT likewise hard to judge. To address this problem, we decided to consider IgRT only part of granulomatous disease treatment regimen if it was clearly stated by the authors of the included article, or when IgRT was started simultaneously with other treatment for granulomatous disease as part of the treatment regimen. IgRT was sometimes given as monotherapy, but also in combination with e.g. steroids. Regarding previous work concerning IgRT in CVID, several studies have been published. A beneficial role of IgRT for AI complications has been illustrated by Wang et al., as they observed less events of recurring autoimmune hemolytic anemia (AIHA) and/or immune thrombocytopenic purpura (ITP) after IgRT was initiated (22). However, the role of IgRT for granulomatous disease remains debatable. Within our included case reports, some authors stated IgRT to be beneficial for granulomatous disease (70–72, 75–77). On the other hand, the large study performed by Mechanic et al. did not report an effect of intravenous IgRT on granulomatous disease (7). Although it has to be mentioned that some of these patients in the study by Mechanic et al. also received steroids, of which in general no effect on granulomatous disease was reported likewise (7). Taking all this into consideration, we believe IgRT to be an essential part of standard treatment in CVID, of which the effect on granulomatous disease remains to be clarified.

We attempted to elucidate treatment regimens and their efficacy in patients with CVID and granulomatous disease with an undefined genetic background. Although we actively excluded cases where genetic variants were described, we cannot rule out that included cases do have an unreported genetic variant associated with CVID. In an increasing number of patients with CVID, a genetic variant is found (1, 103, 104). In case a genetic variant is known, potential pathogenic pathways could be determined and specific targeted therapies could be considered. As an example, the use of abatacept in patients with LRBA or CTLA4 haploinsufficiency with granulomatous disease is associated with improved clinical outcome, but has not been reported in our analysis (105–107). Other known genetic defects associated with a CVID phenotype, including RAG deficiencies, may also influence therapeutic strategies (108, 109). For various genetically defined CVID patients with GILD, such as CTLA4 or LRBA deficiency, also hematopoietic stem cell transplantation (HSCT) has been described as therapeutic option (110, 111).

LIMITATIONS

Patients suffering from CVID with granulomatous disease, form a heterogeneous and complex subgroup of this primary immunodeficiency with a relatively rare complication. As previously shown over decades, treatment regimens for granulomatous disease are also heterogeneous (8, 23, 96, 112). Only a limited number of manuscripts on the topic could be retrieved. Another limitation is, that mainly case reports or case series were included, which are considered to be of the lowest of scientific evidence. Additionally, it is also likely that mainly case reports in which the treatment was associated with remission of the granulomatous disease are published. Also, we actively excluded literature cases were a genetic variant linked to CVID was reported, thereby perusing to include only genetically undefined CVID patients. However, genetic evaluation might not always be performed in patients from the included articles. Thereby, CVID patients with granulomatous disease and an (unknown) genetic variant might be present in the performed analysis. This is an important consideration to take into account regarding interpretation of our findings. Additionally, it is important to realize that information regarding duration of remission of granulomatous disease by the discussed treatment regimens is not well reported in the majority of the included papers.

FUTURE RECOMMENDATIONS

Ideally, large randomized controlled studies should be performed with a long follow-up period, to objectively determine what are the most effective treatment regimens in CVID+EGD or CVID +PGD. However, due to the limited number of CVID patients with granulomatous complications, setting up such a trial is challenging. International clinical trials should be considered. As illustrated by this review, and by the review of Lamers et al., evidence for deciding which treatment should be applied in granulomatous disease is limited, contains heterogeneous regimens, and is of limited scientific weight. However, currently it seems the best possible way to determine promising treatment options. We believe that the systematic search of literature performed here could provide a valuable tool for clinicians treating patients with granulomatous CVID, especially regarding extrapulmonary involvement. Steroids seem effective in the treatment of CVID+EGD. Although the absolute number of reported targeted therapies, such as infliximab, etanercept, rituximab and azathioprine, are low in the CVID+EGD group, we believe these targeted therapies could be of added value in treating extrapulmonary granulomatous disease in CVID, as has also been described in CVID+PGD.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

AS and HI screened the articles for eligibility, performed analysis, and wrote the paper. VD was involved in screening of

the articles and writing of the paper. WD, LK, BS, JM, and PH gave advice on the results and critically red the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2020.606389/full#supplementary-material

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Granulomatous-Lymphocytic Interstitial Lung Disease in Common Variable Immunodeficiency—Features of CT and ^{18}F-FDG Positron Emission Tomography/CT in Clinically Progressive Disease

*Mai Sasaki Aanensen Fraz[1], Natasha Moe[2], Mona-Elisabeth Revheim[2,3], Maria L. Stavrinou[2], Michael T. Durheim[3,4], Ingvild Nordøy[1], Magnhild Eide Macpherson[1,5], Pål Aukrust[1,3,5], Silje Fjellgård Jørgensen[1,5], Trond Mogens Aaløkken[2,3] and Børre Fevang[1,5,6]**

[1] Section of Clinical Immunology and Infectious Diseases, Oslo University Hospital, Oslo, Norway, [2] Division of Radiology and Nuclear Medicine, Oslo University Hospital, Oslo, Norway, [3] Institute of Clinical Medicine, University of Oslo, Oslo, Norway, [4] Department of Respiratory Medicine, Oslo University Hospital, Oslo, Norway, [5] Research Institute of Internal Medicine, Oslo University Hospital, Oslo, Norway, [6] Centre for Rare Diseases, Oslo University Hospital, Oslo, Norway

Common variable immunodeficiency (CVID) is characterized not only by recurrent bacterial infections, but also autoimmune and inflammatory complications including interstitial lung disease (ILD), referred to as granulomatous-lymphocytic interstitial lung disease (GLILD). Some patients with GLILD have waxing and waning radiologic findings, but preserved pulmonary function, while others progress to end-stage respiratory failure. We reviewed 32 patients with radiological features of GLILD from our Norwegian cohort of CVID patients, including four patients with possible monogenic defects. Nineteen had deteriorating lung function over time, and 13 had stable lung function, as determined by pulmonary function testing of forced vital capacity (FVC), and diffusion capacity of carbon monoxide (DLCO). The overall co-existence of other non-infectious complications was high in our cohort, but the prevalence of these was similar in the two groups. Laboratory findings such as immunoglobulin levels and T- and B-cell subpopulations were also similar in the progressive and stable GLILD patients. Thoracic computer tomography (CT) scans were systematically evaluated and scored for radiologic features of GLILD in all pulmonary segments. Pathologic features were seen in all pulmonary segments, with traction bronchiectasis as the most prominent finding. Patients with progressive disease had significantly higher overall score of pathologic features compared to patients with stable disease, most notably traction bronchiectasis and interlobular septal thickening. 18F-2-fluoro-2-deoxy-D-glucose (^{18}F-FDG) positron emission tomography/CT (PET/CT) was performed in 17 (11 with progressive and six with stable clinical disease) of the 32

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***Correspondence:**
Børre Fevang
borre.fevang@rr-research.no

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patients and analyzed by quantitative evaluation. Patients with progressive disease had significantly higher mean standardized uptake value (SUVmean), metabolic lung volume (MLV) and total lung glycolysis (TLG) as compared to patients with stable disease. Nine patients had received treatment with rituximab for GLILD. There was significant improvement in pathologic features on CT-scans after treatment while there was a variable effect on FVC and DLCO.

Conclusion: Patients with progressive GLILD as defined by deteriorating pulmonary function had significantly greater pathology on pulmonary CT and FDG-PET CT scans as compared to patients with stable disease, with traction bronchiectasis and interlobular septal thickening as prominent features.

Keywords: GLILD, Interstitial lung disease (ILD), Primary immumunodeficiencies, DLCO, rituximab, CVID- Common Variable Immunodeficiency Disorders, Pulmonary CT, FDG – PET

INTRODUCTION

Common variable immunodeficiency (CVID) is the most common symptomatic primary immunodeficiency in adults with a prevalence of 1:50,000–1:25,000 in Caucasians (1). Patients are characterized by decreased levels of immunoglobulin (Ig) G, IgA, and/or IgM, typically resulting in recurrent respiratory infections with encapsulated bacteria (2). Up to 70% of CVID patients also present with non-infectious inflammatory complications (3). Interstitial lung disease (ILD) is a common non-infectious manifestation of CVID, and is associated with increased morbidity and mortality (4). The clinical picture ranges from asymptomatic patients with radiological ILD features only, to patients with chronic respiratory failure in need of lung transplantation. The natural disease course is variable, and there are few known early predictors of a progressive disease course.

The term "granulomatous-lymphocytic interstitial lung disease (GLILD)" was first proposed in 2004 by Bates et al. (4). They categorized a group of CVID patients as having GLILD after histological findings in lung biopsies that included granulomas, lymphoid interstitial pneumonitis, lymphoid hyperplasia, and follicular bronchiolitis. Others have described an even broader and combined pathological spectrum in CVID patients with ILD, with histological findings also including organizing pneumonia, non-specific interstitial pneumonia, and diffuse lymphoid hyperplasia (5–7). These findings could represent variation within a spectrum of benign lymphoproliferative lung pathology, or several different pathophysiological mechanisms (5, 6, 8). However, the need for lung biopsies in GLILD diagnosis is debated (9), and the need for other diagnostic tools with less risk of complications is clearly warranted.

Radiologically, GLILD has been characterized by CT findings such as reticulation, bronchial wall thickening, pulmonary nodules, and ground glass opacities, and CT is widely used in the management of these patients (10, 11). FDG-PET/CT imaging is a promising approach in the evaluation of inflammatory disease and has been reported in case studies of GLILD, but has not been evaluated in a larger cohort (12, 13).

Systemic corticosteroids are considered as first-line treatment in patients with GLILD, but the evidence to support this is limited (9). Rituximab alone or in combination with azathioprine or mycophenolate has been reported effective in some retrospective studies and case reports (7, 13–17). There are also case reports describing positive effects of sirolimus, TNF-inhibitors, methotrexate, hydroxychloroquine, cyclosporine, and mycophenolate alone (18, 19). However, there is no consensus regarding optimal treatment of this disorder and no randomized studies have been performed.

We aimed to further elucidate the roles of non-invasive diagnostic tools in GLILD, and in this retrospective observational study we present clinical, immunological, and radiological (including both CT and FDG PET/CT) features in our cohort of patients with GLILD. We compare these features in patients with stable or progressive clinical disease based on functional pulmonary testing. We also describe lung function trajectory and changes in CT and FDG-PET/CT findings among patients treated with rituximab.

METHODS

Patient Population

Patients were recruited from a cohort of 240 CVID patients that are or have been followed at of the Section of Clinical Immunology and Infectious Diseases at Oslo University Hospital. CVID was defined as having decreased serum levels of IgG, IgA, and/or IgM by a minimum of two standard deviations below the mean for age, while excluding other causes of hypogammaglobulinemia. Written informed consent was obtained from all included patients and the study was approved by the Regional Ethical Committee (REC South-Eastern Norway, no 2012/521 and 33256). Patients with pulmonary CT descriptions suggestive of ILD and/or GLILD in a retrospective screening of their electronic medical record were included.

Clinical and Laboratory Data

Laboratory and clinical data, including data on immunomodulatory treatment, were collected by retrospective review of electronic

medical records. The patients' most recent laboratory data for lymphocyte profile with B- and T-cell subpopulations were registered, and where possible, IgA-, IgM- and IgG-levels measured at the same time point. In patients who had received rituximab or other immunomodulatory treatment for GLILD, the most recent laboratory data prior to this treatment was chosen. In patients receiving intravenous immunoglobulins, immunoglobulins were measured immediately prior to infusion.

Pulmonary Function Tests and Definition of Stable and Progressive Disease

All pulmonary function test (PFT) results including forced vital capacity (FVC) and diffusing capacity for carbon monoxide (DLCO) performed at our clinic from the patient's first visit until April 2020 were registered. By assessing the change over time in pulmonary function tests, we defined a group with progressive GLILD. These had an absolute decline in FVC percent predicted > 10 percentage points (p.p.) and/or DLCO percent predicted >15 p.p. during the follow-up period. Patients who already had FVC percent predicted < 50 and/or DLCO percent predicted < 40 at their first PFT performed at our hospital were also included in this group, as the decline in lung function was assumed to have started prior to follow-up at our hospital. Patients not meeting these criteria for progressive disease were defined as stable.

The patients treated with rituximab were categorized by pre-treatment DLCO percent predicted above or below 55%, a cut-off derived from the ILD-GAP model, a scoring tool that has shown to perform well in predicting mortality in patients with chronic ILD (20).

CT Imaging

We examined the most recent HRCT performed in each of the 32 patients, if possible avoiding CT performed during acute lower airway infections or when the patient received immunomodulatory therapy for any reason. In patients treated with rituximab targeting GLILD we examined the last CT prior to treatment, and also the first CT after the initial dose of rituximab (ranging from 3 to 16 months after the initial dose). The images were reviewed in consensus on a PACS (Picture Archiving and Communication System) screen in random order by two experienced chest radiologists, blinded to the patients´ lung function and clinical condition. All CT examinations except one were done at our institution.

Thin-section CT images were obtained in the supine position during breath-holding and deep inspiration. Supplementary expiratory scans were obtained in nine patients to verify small airways disease. For evaluation of the lung parenchyma and airways we applied thin reconstructed slice thickness (0.9–1.25 mm) with a high-spatial-frequency hard kernel, 2.5 mm contiguous images in the axial, coronal, and sagittal planes were in addition reconstructed with a medium soft algorithm. Tube current settings were adjusted to each patient's weight.

The presence, extent, and distribution of ILD were evaluated. According to the CT criteria of ILD recommended by the Nomenclature Committee of the Fleischner Society, ILD findings include groundglass opacity, airspace consolidation, reticular patterns, and interlobular septal thickening (21), see **Figure 1**. The presence of associated findings was also assessed, such as bronchiectasis and bronchiolectasis, nodules and micronodules, thickening of peribronchovascular interstitium, pleural irregularity, mosaic attenuation pattern, mucus plugging, and air trapping. Subsegmental air trapping comprising less than 5% of the lung parenchyma was considered normal (22). CT detected ILD was defined as reticular pattern; and/or ground glass opacities, and/or consolidations; and/or nodules (except centrilobular distributed micronodules); and/or traction bronchiectasis, whereas CT detected airways disease was defined as bronchiectasis; and/or air trapping; and/or mosaic pattern; and/or centrilobular micronodules.

The extent of ground glass opacities and consolidation in each segment was assigned a score based on the percentage of lung parenchyma involved (0, no involvement; 1, 1 to 4%

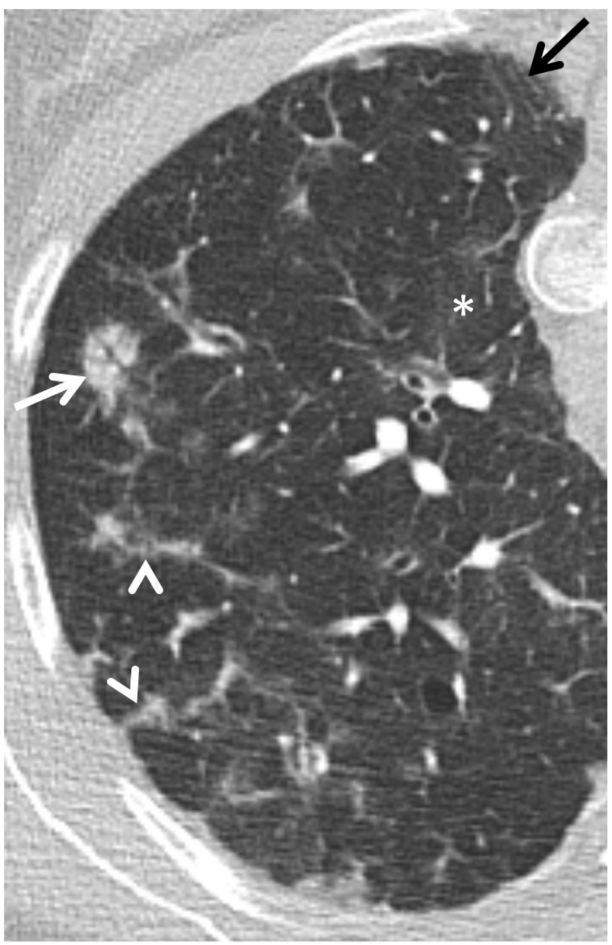

FIGURE 1 | HRCT image of the right upper lobe of a 40-year-old woman with characteristic findings of granulomatous-lymphocytic interstitial lung disease (GLILD) with irregular peribronchovascular interstitial thickening (white arrow), interlobular septal thickening (arrowheads), subtle ground glass opacities (asterix), and traction bronchiectasis (black arrow).

involvement; 2, 5 to 20% involvement; and 3, more than 20% involvement). The severity of traction bronchiectasis was scored 0–3 (1=bronchial wall thickening without distinct ectasias; 2, mild or moderate; and 3, severe bronchiectasis). Nodules, interlobular septal thickening and peribronchovascular interstitial thickening were scored 0–3 (0, absent; 1, mild; 2, moderate; and 3, severe). An overall score of abnormality involvement for each patient was derived by summing the scores of the 18 segments for each finding. Thus, both the overall extent of lung disease (regardless of pattern) and the extent of individual findings were scored, using approximately 45 min evaluating each CT scan.

18F-2-Fluoro-2-Deoxy-D-Glucose PET/CT Imaging

Seventeen patients underwent 18F-2-fluoro-2-deoxy-D-glucose (^{18}F-FDG) positron emission tomography/CT (PET/CT) at our center during the follow-up period. In patients where PET/CT was performed more than once, the most recent was chosen. Three of the nine patients treated with rituximab for GLILD were examined with PET/CT before and after treatment, and these images were compared.

All PET/CT procedures were performed according to the European Association of Nuclear Medicine (EANM) guidelines to ensure comparability between patients, which include quality control, calibration, and harmonization of the scanners and SUV calculations and the PET/CT scans were performed on EARL-accredited (EANM Research Ltd) PET/CT systems GE Discovery 690 (n=16) and [Siemens Biograph 64 (n=5)] (23). The patients fasted for at least 6 h, and blood samples were obtained to document blood glucose levels (median 5.0 mmol/L, range 4.2–9.1 mmol/L) prior to intravenous administration of median 186 MBq ^{18}F-FDG (range 120–296 MBq) and median 370 MBq ^{18}F-FDG (range 233–404 MBq) for the GE Discovery and Siemens Biograph scanners, respectively. Images were obtained approximately 60–90 min. post-injection (median 70, range 63-115 min). A low-dose CT scan was performed and followed by a 3D PET scan using a whole-body acquisition protocol from the vertex to below the knee. PET acquisition times were 2.5 min/field of view (FOV) for the GE Discovery scanner and 3 min/FOV Siemens Biograph scanner.

Quantitative PET Image Evaluation

The primary analysis of the ^{18}F-FDG PET/CT images was conducted by individual image evaluation using PMOD software (PMOD Technologies LLC, version 3.510). To obtain regions of interest (ROI) in the lung, transverse slices of the fused PET/CT images were manually contoured from the apex to the base of both lungs (slice thickness of 2.79mm). Surrounding structures, including hilar regions, were excluded. The mean standardized uptake value (SUVmean), the maximum standardized uptake value (SUVmax), and lung volumes were calculated by the software. An adaptive thresholding algorithm defining a threshold of 41% of the SUVmax–SUVmin measured the metabolic lung volume (MLV) (24, 25). Total lung glycolysis (TLG) was calculated by multiplying MLV with SUVmean of MLV.

Statistics

Associations between stable or progressive GLILD and categorical clinical parameters were assessed by chi square tests. Differences in continuous variables between two groups were analyzed using non-parametric Mann Whitney tests. Paired samples were analyzed using the Wilcoxon rank sum test. Changes in DLCO and FVC before and after treatment with rituximab were analyzed comparing the last value before the first treatment with the best available value after treatment. Annual rate of change in percent predicted DLCO was calculated by linear regression analysis. Kruskal-Wallis test was used to analyze differences between more than two groups. All tests were two-sided with a significance level of 0.05.

RESULTS

Patient Characteristics

We identified 35 patients with CTs suggestive of ILD. After review by two chest radiologists, three of these patients were deemed not likely to have GLILD and were excluded from the study, leaving 32 patients with radiologic features consistent with GLILD. The patients are characterized in **Table 1**. Two patients in our cohort have been diagnosed with lymphoma, diagnosed and treated after data registry for this study. Of other malignancies in this cohort, two were treated for breast cancer and one for prostate cancer. Three of the patients were deceased (at age 36, 48, and 73). Median follow-up time was 123 months (IQR 40–156). Four of the 32 patients had a possible monogenic defect with a known association to CVID, two of these patients were in the progressive group [CTLA4-haploinsufficieny not previously described variant but likely pathogenic; STAT3 variant of uncertain significance (VUS)] and two in the stable group (NFkB1 and BACH2, both VUS). Five of the patients in our cohort had lung biopsy performed, all transbronchial. Only one of these revealed granulomas; the other four showed non-specific inflammation.

Stable and Progressive Clinical Disease

Nineteen patients (59%) were found to have progressive GLILD and 13 (41%) to have stable GLILD. The stable and the progressive group were similar with respect to gender, age, history of smoking, and co-existing obstructive lung disease. The median follow-up time, however, was shorter in the stable than the progressive group (73 *vs.* 142 months, respectively. p=0.033). Importantly, we found no significant difference in initial FVC or DLCO between patients who later developed progressive versus stable disease.

TABLE 1 | Patient characteristics.

	All patients (n = 32)	Stable disease (n = 13)	Progressive disease (n=19)	p-value*
Age (years)**	48 (37–59)	44 (37–56)	51 (39–61)	0.274
Female sex, n (%)	17 (53)	5 (39)	12 (63)	0.169
Known monogenic defect,*** n (%)	4 (13)	2 (15)	2 (11)	0.683
Coexisting obstructive lung disease, n (%)	4 (13)	1 (8)	3 (16)	0.496
History of smoking, n (%)	6 (19)	2 (15)	4 (21)	0.687
First DLCO at our clinic (% of predicted)**	77 (65–85)	81 (65–85)	75 (67–83)	0.828
First FVC at our clinic (% of predicted)**	96 (75–105)	99 (90–109)	82 (69–105)	0.172
Follow-up time (months)**	123 (40–156)	73 (15–74)	142 (59–157)	0.033
Other non-infectious complications				
Lymphadenopathy, n (%)	30 (94)	11 (85)	19 (100)	0.077
Splenomegaly, n (%)	29 (91)	12 (92)	17 (90)	0.787
CVID associated enteropathy, n (%)	14 (44)	5 (39)	9 (47)	0.618
Autoimmune cytopenia, n (%)	12 (38)	6 (46)	6 (32)	0.403
Granulomas in other tissue, n (%)	12 (38)	5 (39)	7 (37)	0.926
NRH in liver, n (%)	8 (25)	3 (23)	5 (26)	0.835
Immunoglobulin substitution form§				
IVIG, n (%)	11 (34)	2 (15)	9 (47)	0.061
SCIG, n (%)	18 (56)	7 (54)	11 (58)	0.821
fSCIG, n (%)	5 (16)	3 (23)	2 (11)	0.337
Immunomodulatory treatment for GLILD				
Any treatment (%)	12 (38)	2 (15)	10 (53)	0.033
Rituximab (%)	8 (25)	1 (8)	7 (37)	0.034
Corticosteroids (%)	8 (25)	2 (15)	6 (32)	0.300
Azathioprine (%)	7 (22)	0 (0)	7 (37)	0.013
Abatacept (%)	1 (3)	0 (0)	1 (5)	0.401
Anti TNF agents (%)	1 (3)	0 (0)	1 (5)	0.401
Immunomodulatory treatment, other indications				
Rituximab (%)	4 (13)	2 (15)	2 (11)	0.683
Corticosteroids (%)	15 (47)	7 (54)	8 (42)	0.513

Stable and progressive disease compared.

**Median and interquartile range.*

***Whole exome sequencing performed in 29/32 patients.*

§*IVIG, intravenous immunoglobulins; SCIG, subcutaneous immunoglobulins; fSCIG, fascilitated SCIG.*

Co-Existing Non-Infectious Complications

The majority of GLILD patients had splenomegaly (91%) and lymphadenopathy (94%). Also, a considerable proportion had had autoimmune cytopenias (38%), 25% had liver disease with biopsy verified nodular regenerative hyperplasia (NRH), 44% had biopsy verified CVID associated enteropathy, and 38% had granulomas in other tissue. We found no difference in the prevalence of co-existing non-infectious complications between the stable and the progressive GLILD group.

Immunological Parameters

The median fraction of class-switched B-cells and plasmablasts in our total GLILD-cohort were 0.8% (normal range 4.3–23.0%), and 0.0% (normal range 0.3–5.1%), respectively (**Table 2**). The median fraction of $CD21^{low}$ B-cells was 19.1% (normal range 1.2–9.4%). Four patients had a fraction of class-switched B-cells > 70% of lower limit of normal range. Patients were overall adequately substituted with immunoglobulins with median serum IgG concentration at 8.75 g/L. Twenty-seven of the 32 patients had not detectable levels of IgA. There were no differences in T- or B-cell subpopulation proportions, nor differences in IgG-, IgA-, or IgM-levels between the stable and progressive GLILD group. Also, the change in IgM-levels from the time point of the first PFT performed at our center to the last, or the last before GLILD directed therapy in patients receiving this, was not significantly different in the two groups.

CT Findings

The most recent CT in each patient (in patients receiving rituximab the most recent CT prior to treatment) was scored. Traction bronchiectasis had the highest overall score of the predefined pathological radiological features, while interlobular septal thickening, ground glass opacities and peribronchovascular interstitial thickening were also frequent findings (**Figure 2A**). ILD-related pathology was present in all lobes and segments, with significantly lower scores in some of the apical segments as compared to basal segments (**Figure 2B**).

Comparing patients with stable and progressive clinical disease, we found a significantly greater total pulmonary CT pathology in the group with progressive disease, most notably interlobular septal thickening (**Figure 3**, **Supplementary Figure 1**). Patients with progressive disease also had significantly higher score of traction bronchiectasis associated with interstitial lung disease than patients with stable disease. In addition, patients with progressive disease had increased features of overall pulmonary CT pathology in all lobes compared to patients with stable disease (**Figure 2C**). In contrast, we could not

TABLE 2 | Laboratory data.

T- and B-cells with subpopulations*	Normal range	All patients (n = 32)	Stable disease (n = 13)	Progressive disease (n=19)
Total T-cells (x 10^6/L)	800–2,400	1120 (724–1,503)	1130 (751–1,333)	966 (722–1,554)
CD4+ T-cells (x 10^6/L)	500–1,400	554 (376–729)	555 (409–748)	553 (296–721)
CD8+ T-cells (x 10^6/L)	200–1,000	465 (252–796)	498 (257–691)	365 (238–903)
% Follicular CD4+ T-cells	6.2–18.0	24.4 (17.3–31.4)	24.1 (17.5–29.7)	24.7 (17.1–36.0)
% Naive CD4+ T-cells	25.0–71.0	21.0 (12.5–31.8)	22.0 (16.0–30.2)	20.6 (11.6–35.4)
% Naive CD8+ T-cells	34.0–87.0	30.2 (17.5–41.1)	28.5 (15.–34.8)	33.2 (17.9–43.3)
% CD8+ early effector T-cells	2.9–16.0	15.5 (10.9–23.4)	12.0 (10.9–40.8)	18.9 (9.8–52.5)
% CD8+ late effector T-cells	2.6–58.0	49.5 (26.0–67.0)	58.7 (31.1–71.0)	41.3 (25.0–67.0)
% T_{reg}	2.5–5.8	2.8 (2.0–3.6)	2.5 (1.9–3.2)	3.0 (2.1–4.0)
Total B-cells (x 10^6/L)	100–500	90 (20–225)	107 (15–345)	66 (24–195)
% Class switched B-cells**	4.3–23.0	0.8 (0.5–1.7) *(n =27)*	0.7 (0.5–1.3) *(n = 10)*	0.8 (0.3–2.6) *(n = 17)*
% Transitional B-cells**	0.6–4.6	5.3 (2.1–12.9) *(n = 27)*	6.0 (4.0–14.0) *(n = 10)*	4.7 (2.0–12.8) *(n = 17)*
% Plasmablasts**	0.3–5.1	0.0 (0.0–0.0) *(n = 27)*	0.0 (0–0) *(n = 10)*	0.0 (0–0.05) *(n = 17)*
% $CD21_{low}$ B-cells**	1.2–9.4	19.1 (8.9–36.6) *(n = 26)*	15.35 (8.3–27.8) *(n= 10)*	21.5 (11.8–41.4) *(n = 16)*
Immunoglobulin levels*				
IgG (g/L)	6.1–14.9	8.75 (7.53–10.05)	9.20 (6.25–10.45)	8.70 (7.60–9.30)
IgM (g/L)	0.7–4.3	0.15 (0.00–0.44)	0.12(0.00–0.30)	0.18 (0.00–1.30)
IgA (g/L)	0.4–2.1	0.00 (0.00–0.00)	0.00 (0.00–0.12)	0.00 (0.00–0.00)
ΔIgM during follow-up***		0.00 (0.00–0.34)	0.00 (–0.03–0.34)	0.00 (0.00–0.56)

Median and interquartile range.

***Class-switched B-cells, transitional B-cells and plasmablasts were analyzed in 27 patients, $CD21_{low}$ B-cells were analyzed in 26 patients.*

****No statistically significant change in IgM between stable and progressive group.*

detect significant differences in scores of the specific features: ground glass opacities, airspace consolidations, nodules, peribronchovascular and fibrous peribronchovascular interstitial thickening between patients with stable and progressive clinical disease. ROC analyses showed that a threshold of 100 had a sensitivity and specificity for predicting progressive disease at 0.64 and 0.71, respectively (**Supplementary Figure 2**).

Omitting data on the four patients with possible monogenic disease did not significantly alter these CT-findings, with the exception of traction bronchiectasis that no longer differed between the stable and progressive group (**Supplementary Figures 3** and **4**).

PET/CT Findings

^{18}F-FDG PET/CT was performed in a subgroup of the GLILD cohort with six patients with stable and eleven patients with progressive disease. Patients with progressive disease had significantly higher SUVmean in the lungs as compared to patients with stable disease (**Figure 4A**). A similar pattern was seen for MLV and TLG, while SUVmax did not significantly differ between the two patient groups. Omitting data on patients with possible monogenic disease the above-mentioned differences were non-significant (**Supplementary Figure 3**).

Immunomodulatory Treatment

Twelve (37.5%) of our patients had received immunomodulatory treatment targeting GLILD at any time while followed at our clinic. Nine patients had been treated with rituximab, six with prednisolone, seven with azathioprine, one with abatacept, and one with adalimumab. Four patients received rituximab and 15 patients were treated with corticosteroids for other inflammatory complications than GLILD during follow up (**Table 1**).

The nine patients treated with rituximab targeting GLILD received two infusions of 1 g rituximab intravenously 2 weeks apart, every 6 months depending on treatment response. The rituximab treatment was given as monotherapy in two patients, and was combined with 100–200 mg azathioprine in seven patients, however two of these discontinued azathioprine within the three first months. Four of the seven patients that received azathioprine also received a small dose of prednisolone (5–10 mg). Eight of the nine patients treated with rituximab classified as having progressive disease.

Longitudinal measurements of DLCO and FVC for the patients treated with rituximab are shown in **Figure 5**. We found a significant fall in both DLCO and FVC prior to treatment with rituximab (p=0.004 and p=0.004, respectively). Overall, for the nine patients treated with rituximab, there was no significant change after treatment in % predicted DLCO or % predicted FVC. Four patients had a more preserved pre-treatment DLCO with respect to the established ILD-GAP risk stratification model, namely > 55% of predicted. These four patients had a higher annual rate of increase in percent predicted DLCO after treatment than the five with more impaired DLCO (p=0.016). We did not find any effect of rituximab treatment on levels of CD3+, CD4+, or CD8+ lymphocytes, nor levels of IgM or IgA (data not shown).

CT scans performed 6–18 months after the initial dose of rituximab were scored and compared to the most recent pretreatment CT (available in eight patients). We found a

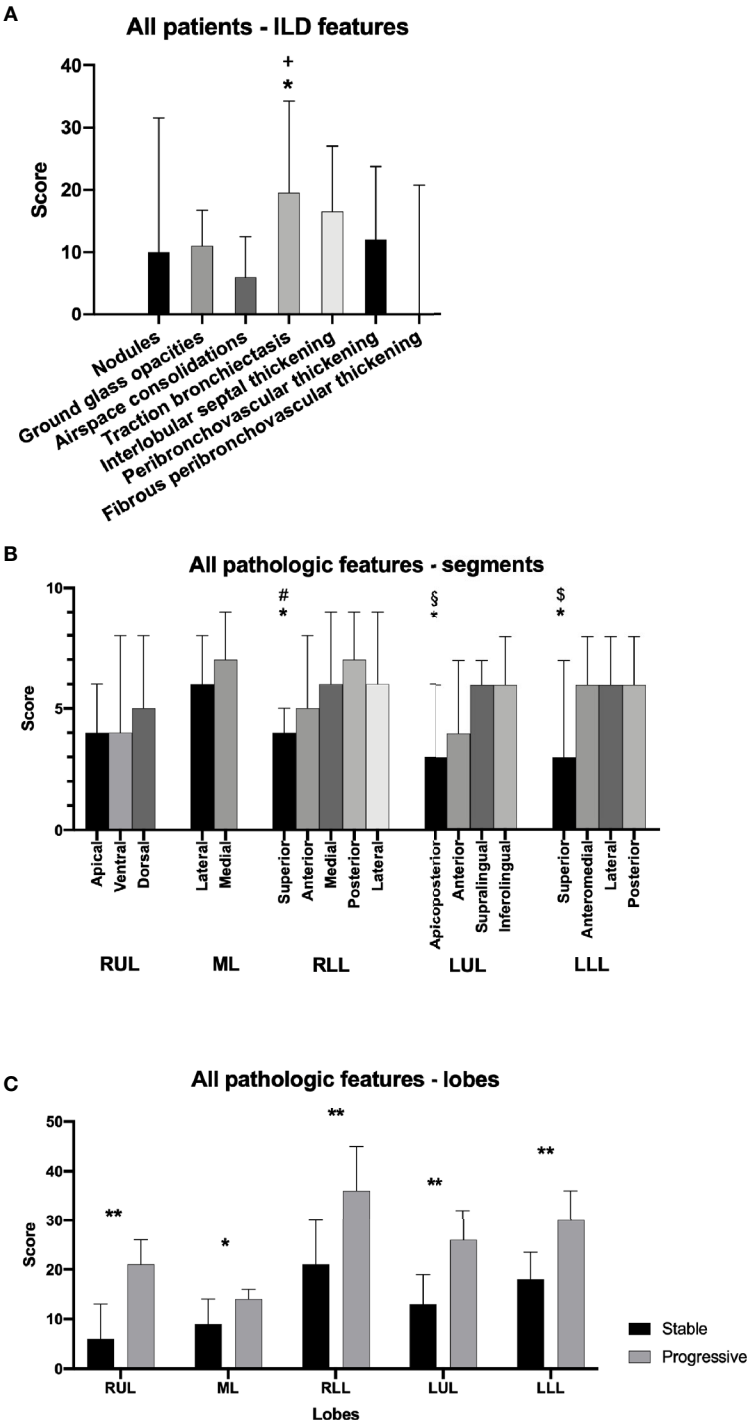

FIGURE 2 | Pathologic features on pulmonary CT scans in granulomatous-lymphocytic interstitial lung disease (GLILD) patients. Overall score for specific features in all patients **(A)**. Overall score of pathologic features in all pulmonary segments for all patients **(B)**. Overall score of pathological features in single lobes in patients with stable and progressive disease **(C)**. RUL, right upper lobe; ML, middle lobe; RLL, right lower lobe; LUL, left upper lobe; LLL, left lower lobe. Median and interquartile range. *p<0.05. **p<0.01. [+]traction bronchiectasis *vs*. ground glass opacities, nodules, consolidations, fibro-/peribronchovascular thickening. [#]superior *vs*. lateral. [§]apicoposterior *vs*. inferolingual. [$]superior *vs*. posterior.

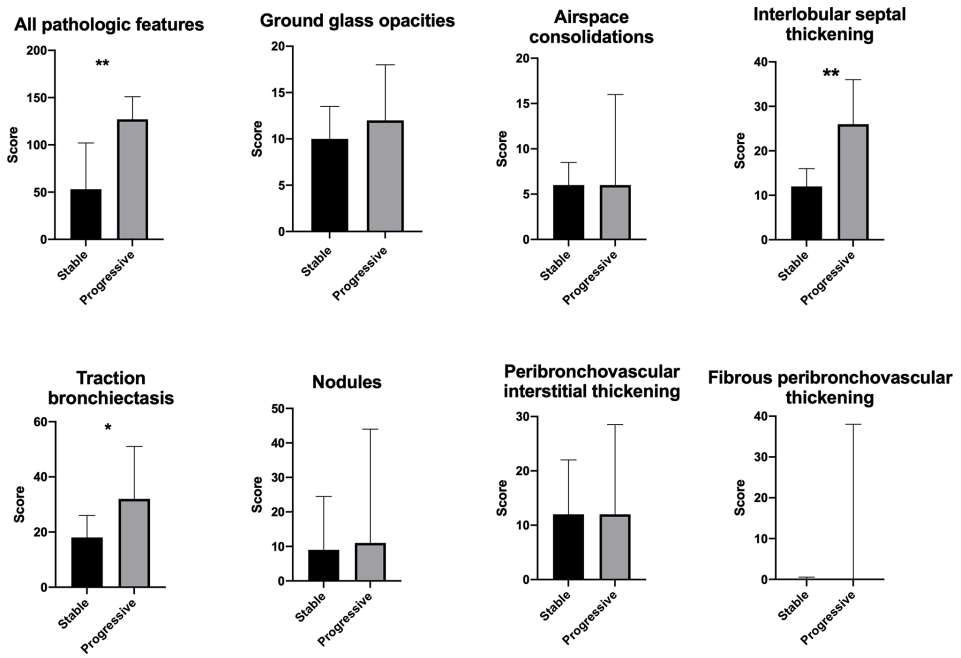

FIGURE 3 | Pulmonary CT scans of patients with stable and progressive disease with score of all pathological features combined and score of specific features. Median and interquartile range. *p < 0.05. **p < 0.01.

significant reduction in overall pulmonary pathology after rituximab treatment, and this improvement was present in all lobes (**Figure 6**). Comparing the extent of the ILD specific radiological features separately before and after treatment, with the exception of interlobular septal thickening changes in each of these were not significant (changes in peribronchovascular interstitial thickening and fibrous peribronchovascular interstitial thickening not shown) (**Figure 6**). Omitting data on patients with possible monogenic disease did not significantly alter these findings (**Supplementary Figure 6**).

Three patients were evaluated with ^{18}F-FDG PET/CT before and after treatment with rituximab. There was a decline in SUVmean, SUVmax, MLV, and TLG for all three patients after treatment (**Figure 4B**, data on SUVmax not shown; **Figure 7**).

DISCUSSION

In this retrospective study of 32 CVID patients with GLILD, we found that patients with clinical progression based on pulmonary functional tests had a significantly greater extent of ILD features on thoracic CT, and more prominent pulmonary inflammation in ^{18}F-FDG PET/CT than those with stable clinical disease. Most notably, patients with progressive clinical disease had a greater extent of traction bronchiectasis and interlobular septal thickening.

In our cohort, 19 of 32 patients had progressive clinical disease, comparable to another previously described cohort (26). Progressive disease can be defined by an absolute decline in pulmonary function but also by decline per time, and we have used the former definition in our study. However, existing data on non-invasive parameters associated with clinical disease progression are scarce. Herein we show that the systematic scoring of pulmonary pathology on CT scans and ^{18}F-FDG PET/CT characteristics could be important diagnostic tools when evaluating disease progression and treatment response in CVID patients with GLILD.

Histopathological features of GLILD may include features of LIP and follicular bronchiolitis (4, 6). Typical CT findings of LIP include ground-glass opacities, bronchovascular bundle thickening (which is similar to peribronchovascular interstitial thickening in our study), and mild interlobular septal thickening, which are overlapping with the CT findings in our GLILD cohort (5, 11, 27). Several GLILD patients had architectural remodeling with traction bronchiectasis, which is a typical finding in ILD and, notably, the presence of this finding was significantly higher in the patients with clinical progression of GLILD. Moreover, interlobular septal thickening and traction bronchiectasis discriminated most clearly between those with and without clinical progression. In contrast, several other features of both LIP and follicular bronchiolitis such as cysts, poorly defined centrilobular nodules and small subpleural nodules, were uncommon findings in our patients. Likewise, intralobular reticular patterns and honeycombing typically seen in fibrotic non-specific interstitial pneumonia (NSIP) and unspecific interstitial pneumonia (UIP) were not identified. These findings may suggest that ILD in CVID patients has other characteristics, and potentially also represents different

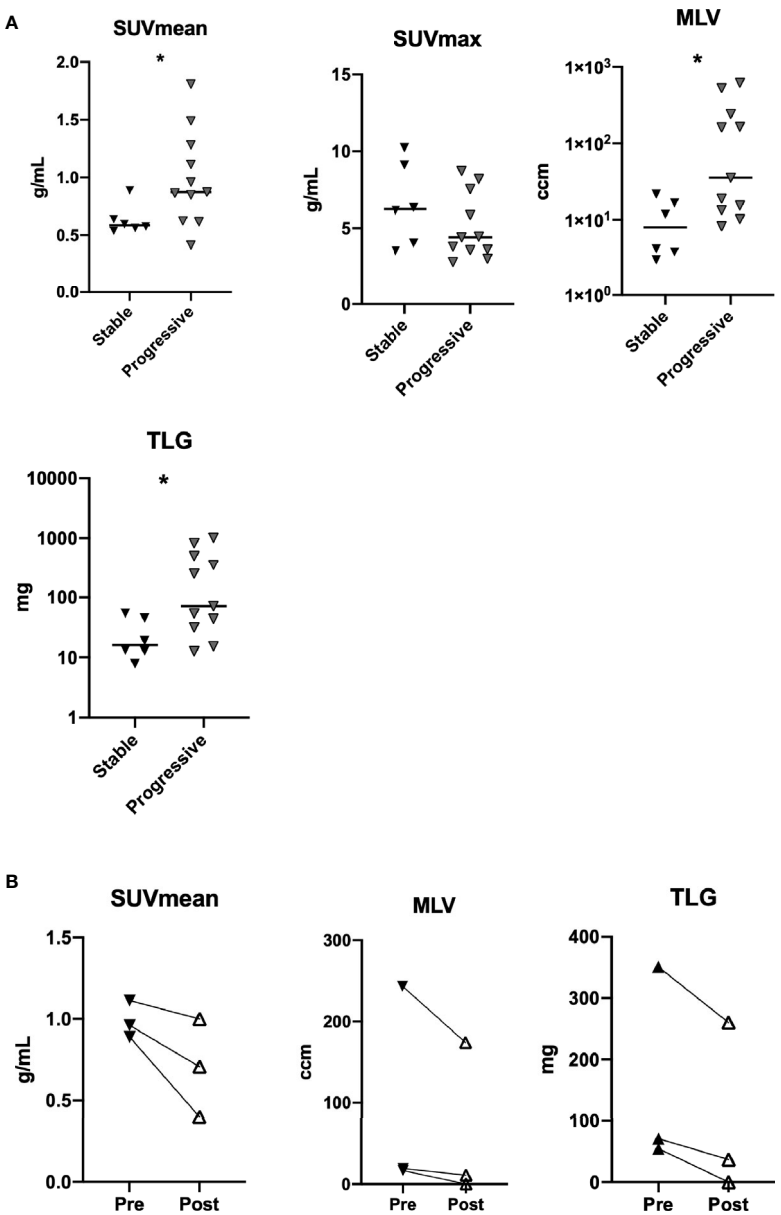

FIGURE 4 | FDG PET-CT in patients with stable and progressive disease as evaluated by SUVmean, SUVmax, metabolic lung volume (MLV), and total lung glycolysis (TLG) (n=17) **(A)**. SUVmean, MLV, and TLG in patients before and after treatment with rituximab (n=3) **(B)**. *$p < 0.05$.

pathophysiological mechanisms than ILD in patients without underlying immunodeficiency. However, these important issues will have to be studied in larger prospective cohorts of CVID patients with GLILD.

Previous data on the use of ^{18}F-FDG PET/CT in evaluating GLILD in CVID patients are scarce, but our data suggest that this could be a valuable tool in the management of GLILD. Indeed, our data showed a significantly higher SUVmean, MLV, and TLG in patients with progressive disease. The SUVmean and volume based MLV and TLG have recently shown to be better prognostic indicators than SUVmax in several studies (28, 29). SUVmax represents the value from one single voxel and does not quantify the total inflammatory burden such as SUVmean, MLV, and TLG (25). Furthermore, a single SUVmax measurement can be unreliable, especially when glucose uptake is heterogeneous and the disease is systemic with multiple lesions such as in GLILD. Thus, SUVmean, MLV, and TLG can provide sensitive and specific values that give insight to the stage and progression of the disease. ^{18}F-FDG PET/CT could therefore be used to identify patients with active pulmonary inflammation and

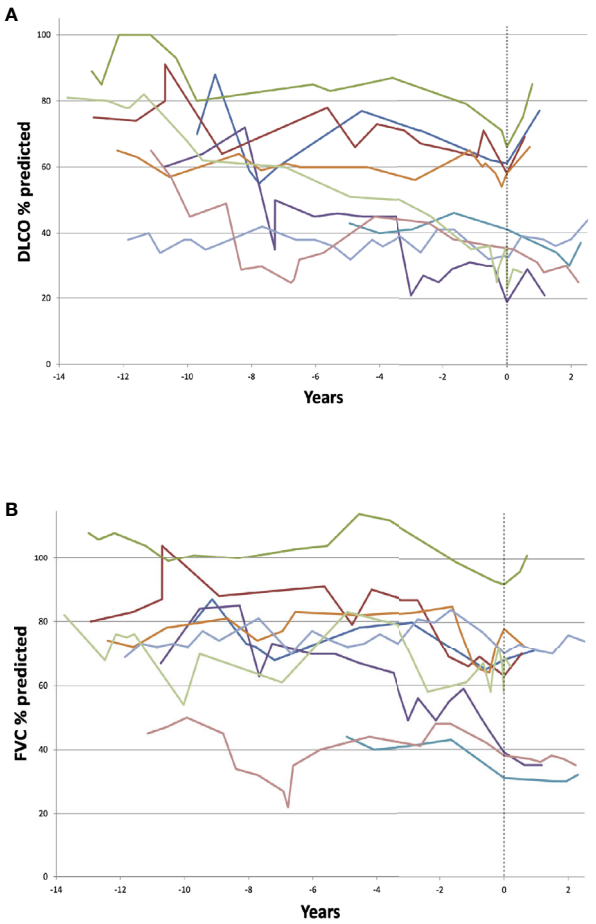

FIGURE 5 | Timeline of diffusion capacity of carbon monoxide (DLCO) **(A)** and FVC **(B)** in nine individual granulomatous-lymphocytic interstitial lung disease (GLILD)-patients treated with rituximab. Dotted line represents time of first rituximab treatment. There was no significant change after treatment in % predicted DLCO or forced vital capacity (FVC). The four patients with a more preserved pre-treatment DLCO with (> 55% of predicted) had a higher annual rate of increase in percent predicted DLCO after treatment than the rest (p=0.016).

progressive disease, as well as evaluate therapeutic measures with a quantitative analysis. In this study we focused on ^{18}F-FDG PET/CT imaging of the lungs only. However, a measurement of the total inflammatory burden, by total body FDG uptake in these patients would be of interest, and subject for future studies.

In contrast to CT and FDG PET-CT, magnetic resonance imaging (MRI) have the advantage of using non-ionizing radiation but has not been systematically evaluated for follow-up of interstitial lung disease (30).

Rituximab has emerged as a preferred second-line treatment for GLILD in combination with immunomodulatory agents. In this retrospective study we included nine patients that were treated with rituximab. As others have reported, overall pulmonary pathology on CT improved clearly after treatment with rituximab (7, 14, 15, 17). There was a generalized pattern of improvement in all lobes, but no change in specific features reached statistical significance, possibly due to low number of patients treated. Furthermore, treatment with rituximab alone or in combination with azathioprine or mycophenolate has been shown to improve functional tests such as FVC and DLCO (7, 13, 15–17). In our nine patients, we did not find any significant change in either DLCO or FVC after rituximab treatment, but the subgroup of four patients with a relatively preserved pre-treatment DLCO (> 55% predicted), showed a greater annual increase in percent predicted DLCO than the remaining five with lower pre-treatment DLCO. This heterogeneity and the small number of patients may explain the discrepancy between changes in CT and PFT. The question of when to start treatment of GLILD is difficult and unanswered, but this observation argues for early initiation of treatment. However, the small number of patients here does not allow for any absolute conclusions.

Patients had similar levels of IgG after substitution and comparable substitution regimens, suggesting that the mode of immunoglobulin substitution has no major influence on GLILD progression, even if it has been claimed that IVIG has immunomodulatory properties that could be beneficial in inflammatory complications of CVID.

Considering the lack of a universally accepted definition of CVID, for the purpose of this study, we found it appropriate to use a broad definition to include patients that we recognize, monitor and treat as CVID with GLILD (2, 31). Four of the patients in our cohort did not fulfill the ESID 2019 definition of smB-cells < 70% of lower limit of normal range (32). We did not have documentation of poor vaccine antibody response in these. All the patients in our cohort had low levels of IgA, and the other "ESID 2019" criteria were met to fulfil the diagnosis.

The present study has several limitations such as its retrospective nature. The lack of longitudinal data on most of the parameters, a low number of patients in the observational rituximab sub-study and a relatively short follow-up time after rituximab treatment are also important limitations. The follow-up time was shorter in the stable group, limiting this study since the definition of progression is partly dependent on observation time. However, the fact that the age of the patients in the two groups were similar, and that we also included patients with pathological pulmonary function tests at first visit at our center is a compensating factor. The data from the patients treated with rituximab should be interpreted with caution based on the low number of patients and the retrospective observational design of the study. CT scans were evaluated qualitatively and even if this were by independent experienced radiologists the lack of quantitative analyses is a limitation of the study. The lack of exercise tolerance test data, data on self-reported dyspnea and frequency of airway infections in this cohort are further limitations of this study. Four of the patients had possible monogenic defects, including one patient with a likely CTLA4-haploinsufficiency, but these were evenly distributed in the two groups.

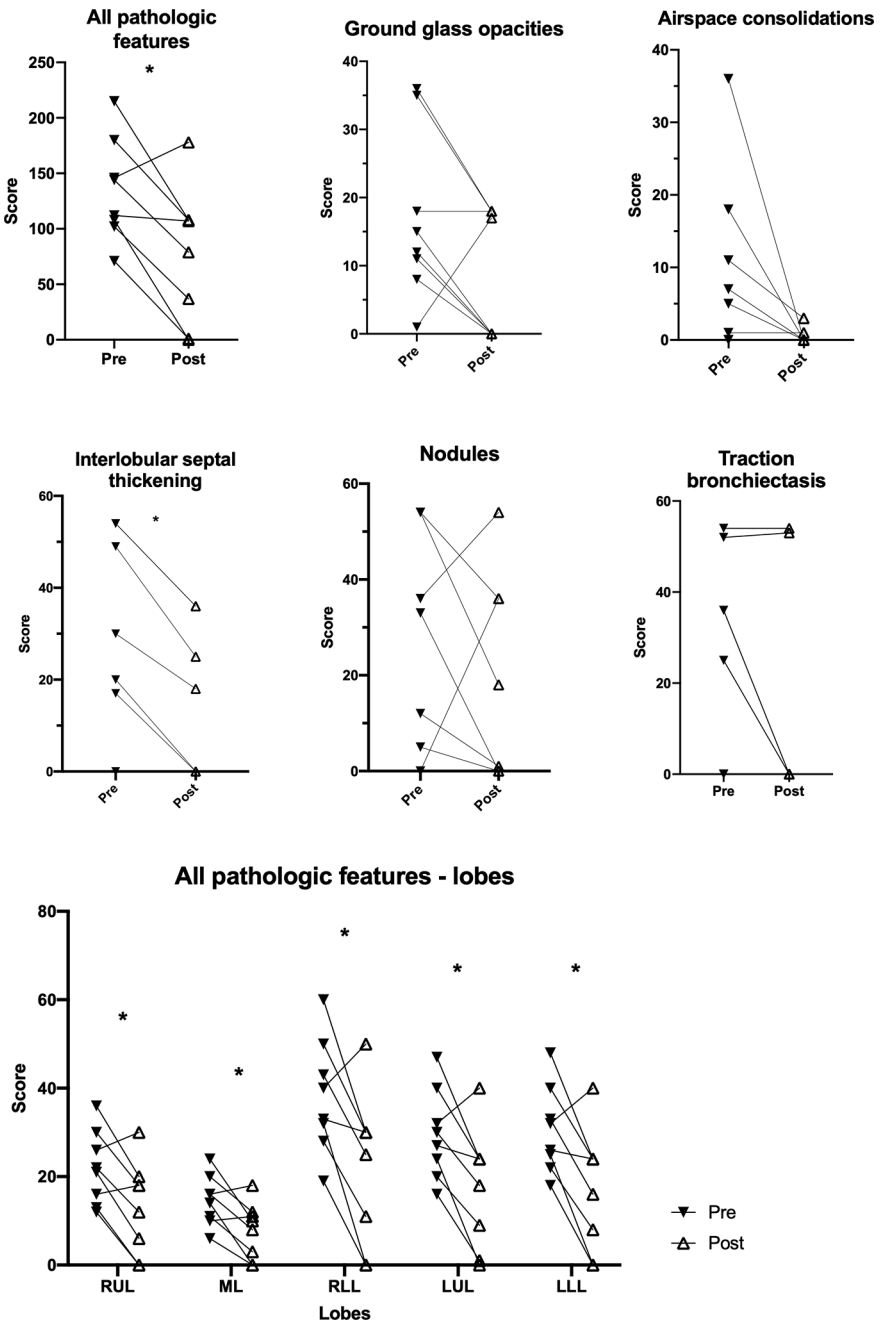

FIGURE 6 | Change in pathological features on pulmonary CT scans in granulomatous-lymphocytic interstitial lung disease (GLILD) patients before and after treatment with rituximab. RUL, right upper lobe; ML, middle lobe; RLL, right lower lobe; LUL, left upper lobe; LLL, left lower lobe. Median and interquartile range. *$p < 0.05$.

CONCLUSION

In this study of 32 CVID-patients with radiological features consistent with GLILD, we found that a majority of patients had progressive disease defined by a decline in PFT results over time. We found a significantly higher overall CT pathology score in patients with progressive GLILD compared to patients with stable GLILD, with interlobular septal thickening and traction bronchiectasis as the most prominent findings. Patients with progressive disease furthermore had significantly higher SUVmean, MLV, and TLG on FDG-PET/CT suggesting that this modality may be valuable for identifying patients with active pulmonary inflammation and

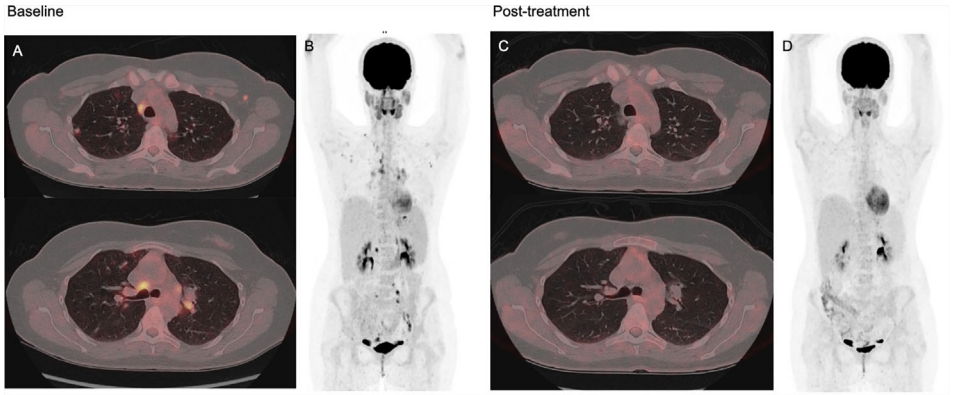

FIGURE 7 | FDG PET/CT at baseline and 3–4 months after monotherapy with rituximab in a 37-year-old common variable immunodeficiency (CVID) patient with granulomatous-lymphocytic interstitial lung disease (GLILD) and generalized lymphadenopathy. Image **(A)** shows axial fused PET/CT images at two different thoracic levels at baseline, with scattered nodular and confluent consolidations in the pulmonary parenchyma with moderate to high FDG uptake, and also moderate to high FDG uptake in mediastinal and hilar lymph nodes. Image **(B)** shows baseline maximum intensity projection (MIP) showing pathologic FDG uptake in lung parenchyma and lymph nodes over and under the diaphragm. Image **(C, D)** shows post-treatment axial fused FDG PET/CT and MIP with complete resolution of both pulmonary and lymph node pathology. Spleen size was within normal range both before and after treatment.

progressive disease, thus complementing CT as a tool in the evaluation of when to start treatment for GLILD. In our cohort, treatment with rituximab was followed by a significant improvement in overall pulmonary CT pathology, while changes in pulmonary function varied. GLILD remains a significant clinical challenge, and identifying factors contributing to disease progression and to clinical improvement following treatment will be important to improve care for these patients.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by REC South-Eastern Norway. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

MSAF, NM, MR, MTD, IN, MEM, PA, SFJ, TMA, and BF designed the study. MSAF, NM, MR, MLS, TMA, and BF analyzed the data. All authors contributed to the writing of the manuscript and read the final version. All authors contributed to the article and approved the submitted version. The publication of this article was made possible through funding from the Norwegian Immunodeficiency Society.

FUNDING

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2020.617985/full#supplementary-material

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An AIREless Breath: Pneumonitis Caused by Impaired Central Immune Tolerance

Elise M. N. Ferré and Michail S. Lionakis *

Fungal Pathogenesis Section, Laboratory of Clinical Immunology and Microbiology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States

Autoimmune-polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED), a monogenic disorder caused by biallelic mutations in the *AIRE* gene, has historically been defined by the development of chronic mucocutaneous candidiasis together with autoimmune endocrinopathies, primarily hypoparathyroidism and adrenal insufficiency. Recent work has drawn attention to the development of life-threatening non-endocrine manifestations such as autoimmune pneumonitis, which has previously been poorly recognized and under-reported. In this review, we present the clinical, radiographic, autoantibody, and pulmonary function abnormalities associated with APECED pneumonitis, we highlight the cellular and molecular basis of the autoimmune attack in the AIRE-deficient lung, and we provide a diagnostic and a therapeutic roadmap for patients with APECED pneumonitis. Beyond APECED, we discuss the relevance and potential broader applicability of these findings to other interstitial lung diseases seen in secondary AIRE deficiency states such as thymoma and RAG deficiency or in common polygenic autoimmune disorders such as idiopathic Sjögren's syndrome.

Keywords: Autoimmune-polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED), autoimmune polyglandular syndrome type-1 (APS-1), autoimmune regulator (AIRE), pneumonitis, interstitial lung disease, bronchiectasis

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John R. Hurst, University College London, United Kingdom

Reviewed by:
Tadashi Ariga, Hokkaido University, Japan
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****Correspondence:***
Michail S. Lionakis
lionakism@mail.nih.gov

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INTRODUCTION

Autoimmune-polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED), also known as autoimmune polyglandular syndrome type-1 (APS-1), is a rare disorder resulting from biallelic mutations in the autoimmune regulator (*AIRE*) gene. AIRE is a thymus-enriched transcription regulator integral for enforcing central immune tolerance. AIRE-deficiency leads to multiorgan system autoimmunity and susceptibility to chronic mucocutaneous candidiasis (CMC). Diagnosis relies on developing two ("diagnostic dyad") out of any three "classic triad" manifestations of CMC, hypoparathyroidism, and adrenal insufficiency. Development of a diagnostic dyad raises suspicion for APECED, which is then confirmed by *AIRE* gene sequencing. Detection of type I interferon (IFN-α/IFN-ω) autoantibodies is sensitive and specific for APECED and is useful for diagnosis (1). While the classic triad is quite characteristic for APECED, exclusive reliance on the classic triad manifestations results in delayed clinical diagnosis as a variety of non-triad non-endocrine manifestations develop often before reaching a classic diagnostic dyad (2). To that end, we have proposed inclusion of an adjunct triad of early-onset manifestations, namely APECED rash,

intestinal dysfunction, and enamel hypoplasia, into expanded diagnostic criteria which would reduce the time to clinical diagnosis by half (3). Establishing an earlier diagnosis is important as it can enable screening for life-threatening endocrinopathies and prompt recognition and treatment of non-endocrine autoimmune manifestations such as hepatitis (4) or pneumonitis (5).

With regard to pneumonitis, prior studies had suggested it to be an uncommon manifestation of APECED (prevalence in all previously-published work, ~2%). A small number of affected patients (2.7–4.5%) had been described among Turkish, Russian, and Indian APECED cohorts. Importantly, the foundational APECED cohort descriptions in Finns, Sardinians, or Iranian Jews do not highlight pneumonitis nor is it a prominent feature in the literature among APECED patients from the British Isles (6–25). In contrast, in a prospective observational natural history study at the NIH, we diagnosed >40% of consecutively-enrolled APECED patients with autoimmune pneumonitis; notably, pneumonitis symptoms presented early in life, often before developing a classic diagnostic dyad (5).

DEFINITION AND CLINICAL PRESENTATION OF AUTOIMMUNE-POLYENDOCRINOPATHY-CANDIDIASIS-ECTODERMAL DYSTROPHY PNEUMONITIS

APECED pneumonitis presents clinically with chronic respiratory symptoms lasting >4 weeks with accompanying radiographic abnormalities of interstitial lung disease (ILD) and/or bronchiectasis. Affected patients most commonly present with daily cough with or without sputum production, and frequently report nocturnal bouts of cough (60%) awakening them from sleep. Less frequently, dyspnea on exertion (57%), pleuritic chest pain (48%), wheezing (43%), and fevers (29%) occur (5). Importantly, a small proportion of patients (<5–10%) is asymptomatic early in the course of pneumonitis (5).

Non-contrast computed tomography (CT) of the chest reveals abnormalities consistent with ILD and/or bronchiectasis. Specifically, ground-glass opacities (GGO) or mosaicism and bronchiectasis are the most common abnormalities; they are seen, either alone or in combination, in all patients with APECED pneumonitis, including those without respiratory symptoms and negative lung-targeted autoantibodies (see below) (5). Additional less common radiographic findings include a tree-in-bud pattern, nodular opacities, and mucus plugging. Taken together, non-contrast chest CT imaging is the most sensitive screening tool for APECED pneumonitis.

In keeping with these chronic symptoms and radiographic abnormalities, APECED pneumonitis leads to abnormal pulmonary function (5, 26, 27). Indeed, affected patients display decreased diffusing capacity of the lungs for carbon monoxide with or without a ventilatory defect by spirometry presenting as obstructive, restrictive, or a mixed pattern of both. A 6 min walk test typically shows decreased walk distance and oxygen desaturation (5).

Progression of Untreated Pneumonitis Causes Morbidity and Mortality

Through the course of our study, we encountered patients across the spectrum of pneumonitis severity which allowed us to characterize the temporal progression of clinical and radiographic features of APECED pneumonitis. Early-stage disease manifests with dry cough associated with GGO and/or a tree-in-bud pattern without bronchiectasis (**Figure 1**). Without immunosuppression, pneumonitis progresses to bronchiectasis-associated structural lung disease presenting with productive cough and bacterial airway colonization. Late-stage untreated pneumonitis features progressively worsening bronchiectasis-associated structural lung disease with development of recurrent infections by Gram-negative bacteria, Gram-positive bacteria, or nontuberculous mycobacteria (NTM) leading to hypoxemia requiring home oxygen therapy (5).

The few clinical cases previously described in the literature corroborate our study observations. DeLuca et al. and Alimohammadi et al. reported a Sicilian child who first developed productive cough and recurrent lower respiratory tract infections at the age of 5 years. The patient's pneumonitis progressed over time with development of a severe obstructive defect, bronchiectasis, chronic airway colonization with *Burkholderia*, and hypoxemia requiring daily oxygen supplementation at the age of 14 years. The patient succumbed to pneumonitis complications when 18 years-old (26, 27). Alimohammadi and colleagues described three additional patients who developed chronic cough in childhood and progressed clinically with recurrent lower respiratory tract infections, an obstructive ventilatory defect, and radiographic evidence of bronchiectasis and/or GGO. One of the patients was oxygen-dependent by 19 years and another died at 37 years from respiratory failure (27).

Therefore, disease progression from symptom onset to end-stage lung disease is highly variable as demonstrated by the aforementioned cases. Similarly, in our recent study we reported a 54-year-old man who developed chronic cough when 5 years-old and progressed over 40 years to eventually develop cavitary pulmonary NTM infection complicated by bronchopulmonary fistula and empyema, chronic hypoxemia requiring daily supplemental oxygen, and death at 56 years. His case stands in contrast to a 14-year-old boy who rapidly progressed from cough onset at 7 years to home oxygen therapy at 11 years and death at 14 years (5).

Therefore, timely diagnosis is necessary to ensure early initiation of immunomodulation in order to arrest progression to bronchiectasis-associated structural lung disease. However, this can be challenging to achieve as symptoms frequently begin in early life and often before the patient develops a classic diagnostic dyad that would raise suspicion for APECED. Even patients with confirmed APECED typically experience delays in pneumonitis diagnosis due to the poor characterization of the entity in the previously-published literature. Consequently, patients are often misdiagnosed with asthma or bronchitis

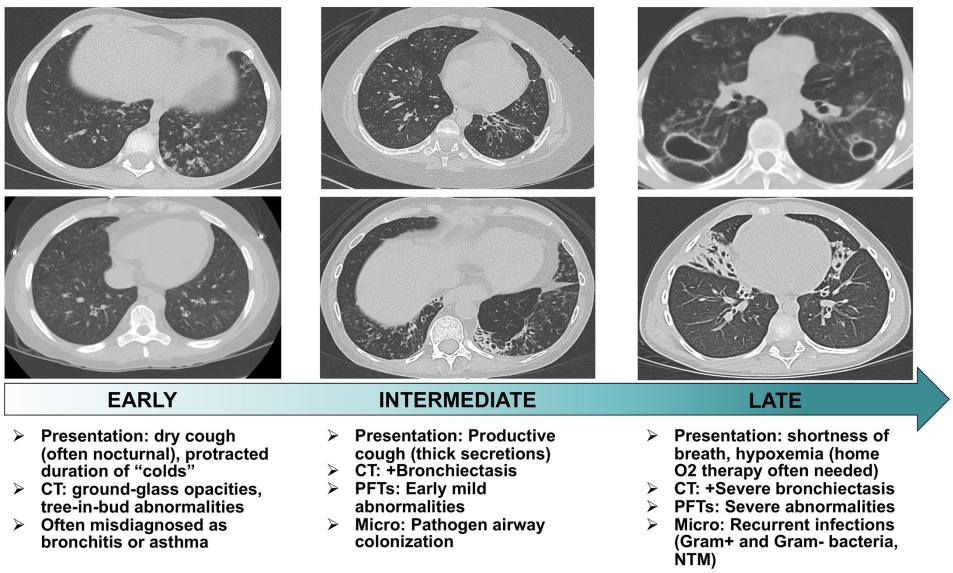

FIGURE 1 | Stages of temporal progression of APECED-associated pneumonitis. Flow chart summarizing the temporal progression of symptoms, radiographic and pulmonary function test abnormalities, and microbiological findings in patients with APECED pneumonitis. CT, computed tomography; PFT, pulmonary function tests; Micro, microbiological findings; NTM, nontuberculous mycobacteria.

resulting in treatment delays thereby increasing the risk of developing structural lung disease and associated morbidity and mortality. For this reason, we recommend that all APECED patients, regardless of symptoms, undergo periodic screening with chest CT to achieve early diagnosis of APECED pneumonitis (5). Moreover, a high index of suspicion for APECED is required by pediatricians and pulmonologists in children who develop chronic respiratory symptoms in the setting of CMC and/or autoimmune manifestations within the classic and/or adjunct diagnostic criteria of APECED.

Pathogenesis of Autoimmune-Polyendocrinopathy-Candidiasis-Ectodermal Dystrophy Pneumonitis

AIRE Genetics and Non-AIRE Modifiers may Impact Pneumonitis Prevalence

APECED is caused by biallelic *AIRE* mutations (28, 29). In our genotype-phenotype analysis, we found an association between carrying the c.967_979del13 mutation in homozygosity with decreased time to development of pneumonitis (5). Autosomal dominant (AD) *AIRE* mutations in the first plant homeodomain (PHD1) zinc finger domain and in the SAND domain have been described to cause organ-specific autoimmune disease resulting in milder phenotypes with reduced penetrance (30–32). While CMC, endocrinopathies and non-endocrine manifestations such as pernicious anemia, nail dystrophy, vitiligo and alopecia have been reported, autoimmune pneumonitis has thus far not been reported in those carrying AD mutations in *AIRE*. The enrichment of the c.967_979del13 mutation in American and British cohorts may explain the differences in prevalence among Americans and British. Alternatively, or in parallel, non-*AIRE* genetic modifiers (33), differential pulmonary microbiome, environmental factors, and/or our unbiased enrollment coupled with a uniform prospective evaluation in all patients regardless of symptoms may contribute to the increased prevalence of pneumonitis among Americans. Future enrollment and uniform multidisciplinary evaluation of European and additional American patients in our and other institutions will be essential to validate our findings.

Thymic Escape of Autoreactive Lymphocytes

AIRE is expressed in thymic medullary epithelial cells (mTECs) where it facilitates the negative selection of self-reactive T-lymphocytes. As a transcription regulator, AIRE promotes the expression of peripheral tissue-restricted antigens on mTECs and the clonal deletion of self-reactive T-lymphocytes; in the AIRE-deficient state, these cells escape in the periphery and are both necessary and sufficient to cause tissue-specific autoimmunity as shown by lymphocyte depletion and adoptive transfer experiments in mice (2, 34–38).

AIRE-deficiency also impairs B-lymphocyte tolerance (39), which contributes to the development of autoimmunity in some, but not all, tissues (40). AIRE-deficient humans and mice produce a broad repertoire of high-affinity autoantibodies (1, 41–44), although these autoantibodies have not demonstrated direct pathogenicity *via* serum transfer studies in mice (37, 40). Instead, B-lymphocytes appear to contribute to autoimmune inflammation through priming effector T-lymphocytes (40).

Several tissue-specific autoantibodies correlate with the development of organ-specific disease in APECED (38, 45–47). Among these, autoantibodies against bactericidal/permeability-increasing fold-containing family B member 1 (BPIFB1) and the potassium channel regulator KCNRG have been associated with development of APECED pneumonitis (3, 21, 27, 48). We

corroborated this finding in our cohort where both autoantibodies were highly specific for pneumonitis and significantly associated with the time to development of pneumonitis (5). Autoantibodies against BPIFB1 were more sensitive compared to those against KCNRG (5). Although the majority (76%) of affected patients carried at least one of these lung-targeted autoantibodies in serum and/or bronchoalveolar lavage (BAL), a quarter of patients with pneumonitis were negative for both autoantibodies. Therefore, while identification of autoantibodies in patient serum may aid as a screening modality of pneumonitis, such testing alone does not suffice to rule out pneumonitis in all individuals, further underscoring the importance of universal screening *via* chest CT imaging. Importantly, these data also underscore the need for future research aimed to identify the lung autoantigens that might be the target of autoimmune attack in patients with APECED pneumonitis who do not carry BPIFB1 or KCNRG autoantibodies.

Autoimmune-Polyendocrinopathy-Candidiasis-Ectodermal Dystrophy Pneumonitis Features a Characteristic Compartmentalized Immunopathology

We performed bronchoscopies in APECED patients with untreated pneumonitis and obtained BAL fluid and endobronchial and transbronchial tissue biopsies for immunological and histological analyses in comparison to healthy volunteer specimens obtained in bronchoscopy. A characteristic compartmentalized immune response was noted, which carries significant diagnostic value. In the airways, an enrichment of neutrophils was seen in the absence of bacterial or other lung infection. In agreement, we observed a significant increase of neutrophil-targeted CXC chemokines in the BAL (CXCL1, CXCL2, IL-8), although the cellular source of these chemokines remains unknown (**Figure 2**). BAL neutrophils exhibited an activated phenotype evidenced by increased expression of the extracellular epitope of the NADPH oxidase b558, of primary, secondary, and tertiary granule contents (CD18, CD63, CD66b), and of CD45, and decreased CD16 expression. Both myeloperoxidase (MPO) and matrix metallopeptidase-9 (MMP-9), products of activated neutrophils, and lactate dehydrogenase (LDH), a surrogate marker of tissue injury, were markedly increased in the BAL fluid of patients with pneumonitis (5). Thus, activated neutrophils appear to contribute to airway tissue injury and may instigate bronchiectasis as postulated in patients with cystic fibrosis and non-cystic fibrosis bronchiectasis (49, 50).

In contrast to the neutrophilic response in the airways, histological examination of endobronchial and deeper lung tissue biopsies demonstrated a chronic inflammatory infiltrate consistent with prior literature describing lymphocytic peribronchiolar inflammation in few patients (**Figure 2**) (5, 26, 27, 51). Endobronchial biopsies from patients with APECED pneumonitis

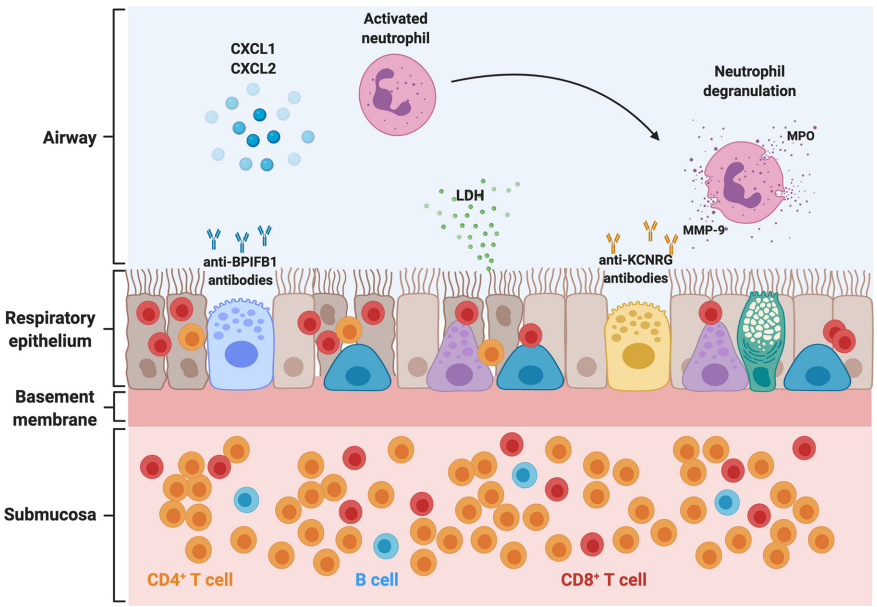

FIGURE 2 | Pathogenesis of APECED-associated pneumonitis. Schematic representation of the abnormalities in the airway, respiratory epithelium, and submucosal tissue in the setting of APECED pneumonitis. T- and B-lymphocytes infiltrate the respiratory tissue. $CD4^+$ T-lymphocytes predominate in the submucosal tissue and peribronchiolar/bronchiolar areas (not depicted), while $CD8^+$ T-lymphocytes display a predominantly intraepithelial distribution. Neutrophils predominate in the airways where they accumulate through the release of CXC chemokines such as CXCL1, CXCL2, and IL-8. Recruited neutrophils acquire an activated phenotype and release MPO and MMP-9 into the airway, which further exacerbates tissue injury, as seen with release of LDH within the airways. Chronic epithelial irritation results in a thickened basement membrane. KCNRG and the BPIFB1 have been identified as bronchial autoantigens targeted by autoimmunity in APECED pneumonitis, and autoantibodies against these targets can be detected in the bronchoalveolar lavage and serum (not depicted) of patients with APECED pneumonitis. BPIFB1, bactericidal/permeability-increasing fold containing family B member 1; LDH, lactate dehydrogenase; MPO, myeloperoxidase; MMP-9, matrix metallopeptidase 9; CXCL1, C-X-C chemokine ligand 1; CXCL2, C-X-C chemokine ligand 2.

displayed a thickened basement membrane with submucosal and intraepithelial lymphocytosis composed predominately of T-lymphocytes with fewer B-lymphocytes. $CD4^+$ T-lymphocytes predominated in the submucosa whereas $CD8^+$ T-lymphocytes were enriched within the intraepithelial compartment (**Figure 2**) (5). No eosinophils or neutrophils were observed infiltrating the tissue. Deeper lung biopsies unveiled lymphocytic or lymphoplasmacytic bronchiolitis and/or peribronchiolar inflammation dominated by $CD4^+$ and $CD8^+$ T-lymphocytes, with mild-to-moderate fibrosis noted in some patients. As with endobronchial biopsy specimens, $CD8^+$ T-lymphocytes predominated within the bronchiolar epithelium while $CD4^+$ T-lymphocytes were prominent in the submucosal bronchiolar tissue. Notably, whereas infiltration of B-lymphocytes was less prominent on endobronchial biopsy specimens, deep peribronchial tissue examination demonstrated marked B-lymphocyte infiltration with development of lymphoid nodules and primary follicles, some of which showed germinal center formation (5).

The mouse model of Aire-deficiency recapitulated the immunological characteristics of autoimmune pneumonitis of patients. Specifically, *Aire*$^{-/-}$ mice exhibited airway neutrophilia with increased neutrophil-targeted CXC chemokines in the absence of an infectious challenge. Moreover, the lung parenchyma of *Aire*$^{-/-}$ mice featured similar histological abnormalities consisting of intraepithelial, submucosal, peribronchiolar and interstitial infiltration composed of T- and B-lymphocytes with B-lymphocyte aggregates observed deeper in the lung tissue (5).

Collectively, APECED pneumonitis features a characteristic pattern of compartmentalized immunopathology consisting of activated neutrophils in the airways with lymphocytic inflammation within the lung parenchyma. This information has important diagnostic value. For example, the presence of neutrophils in the BAL or even in induced sputum examination in an APECED patient with pulmonary symptoms and radiographic abnormalities should raise suspicion for pneumonitis in the absence of pneumonia. Endobronchial biopsies, which we favor as the preferred modality for making a histological diagnosis of pneumonitis, allow for demonstration of intraepithelial and submucosal lymphocytosis, which together with the airway neutrophil expansion provide a high degree of probability for the diagnosis of APECED pneumonitis, especially when combined with BPIFB1- and/or KCNRG-targeted autoantibody positivity.

COMBINATION LYMPHOCYTE-DIRECTED IMMUNOMODULATION REMITS PNEUMONITIS

Previous reports of various immunomodulatory treatments had demonstrated mixed results with one patient responding to T-lymphocyte immunomodulation with azathioprine (27) while other patients required multiple different T-lymphocyte therapies with mixed results (21, 27). Data in the Aire-deficient mouse from our group and others would suggest that a T-lymphocyte depletion approach such as with the CD52-targeting alemtuzumab would remit APECED pneumonitis (5, 37); however, the risk of opportunistic infections makes such T-cell depleting strategies difficult to implement for the lifelong management of pneumonitis (52, 53). Thus, we elected a combination of T-lymphocyte modulation with azathioprine [or mycophenolate mofetil in patients with thiopurine methyltransferase (TPMT) mutations] together with B cell-targeting rituximab to capitalize on the beneficial effects of B-lymphocyte deficiency observed in mice (5). This regimen is used successfully to treat granulomatous and lymphocytic interstitial lung disease (GLILD) seen in combined variable immunodeficiency (CVID) (54).

Combination T and B lymphocyte-directed therapy resulted in resolution of respiratory symptoms in all symptomatic patients within 1 month. Those who had recurrent pulmonary infections secondary to their bronchiectasis before onset of immunomodulatory treatment did not develop infection recurrences after therapy initiation, indicating that the hyper-inflammatory milieu within the untreated airways is permissive for pathogen overgrowth. Immunomodulatory treatment was accompanied by marked improvement of radiographic abnormalities of GGO, tree-in-bud pattern, nodular opacities, and mucus plugging. Improvement was also noted in pulmonary function abnormalities with increased 6 min walk distance and resolution of oxygen desaturation (5). Lymphocyte immunophenotyping showed no changes in $CD3^+$, $CD4^+$, and $CD8^+$ T-lymphocyte numbers in blood and an expected decline in $CD19^+$ B-lymphocytes. Titers of BPIFB1 and KCNRG autoantibodies did not decline despite clinical and radiographic remission of pneumonitis, further suggesting that the pathogenic role of B-cells might be conferred *via* priming of T-cells in the lung tissue, rather than through autoantibody production. This early treatment study of five consecutive patients (5) with pneumonitis and treatment of 6 additional patients with similar results (manuscript in preparation) indicate that combination T and B lymphocyte-directed therapy can remit clinical symptoms and radiographic and functional abnormalities in APECED pneumonitis. Importantly, early initiation of treatment, preferably before the establishment of irreversible bronchiectatic abnormalities, is desirable to avoid the long-term pulmonary complications and morbidity and mortality associated with untreated pneumonitis.

AUTOIMMUNE-POLYENDOCRINOPATHY-CANDIDIASIS-ECTODERMAL DYSTROPHY PNEUMONITIS SHARES IMMUNOLOGICAL FEATURES WITH INTERSTITIAL LUNG DISEASES ASSOCIATED WITH SECONDARY AUTOIMMUNE REGULATOR-DEFICIENCY STATES

Conditions associated with documented secondary AIRE-deficiency in the thymus such as thymoma (55) and inherited

RAG deficiency due to hypomorphic *RAG* mutations that cause delayed onset combined immunodeficiency with granulomas and/or autoimmunity (CID-G/AI) feature autoimmunity and display broad-spectrum autoantibodies against cytokines and tissue autoantigens (56–58) similar to APECED patients. A subset of these patients develops lung disease, which had previously been poorly-characterized (59, 60). We hypothesized that the lung disease seen in patients with thymoma or hypomorphic *RAG* mutations share similar features with APECED pneumonitis. Indeed, thymoma-associated autoimmune lung disease exhibits a similar compartmentalized immunopathology with airway neutrophil expansion and intraepithelial, submucosal, and peri-bronchiolar lymphocytic inflammation as seen in APECED pneumonitis (5). A smaller proportion of these patients carry autoantibodies against BPIFB1 and KCNRG compared to patients with APECED pneumonitis (5), pointing to additional yet-unidentified lung autoantigens in these diseases. Notably, the similarities between autoimmune lung disease seen in the setting of these secondary AIRE-deficiency states and APECED suggest common pathogenetic mechanisms and imply that the lymphocyte-targeted immunomodulatory regimen that is effective in APECED pneumonitis might also remit ILD in patients with thymoma (manuscript in preparation) and may serve as a bridge to hematopoietic stem cell transplantation in patients with ILD in the setting of hypomorphic RAG mutations with CID-G/AI.

Beyond primary and secondary AIRE-deficiency states, ILD with a similar compartmentalized immunopathology consisting of airway neutrophil expansion and lymphocytic bronchiolitis develops among a subset of patients with certain polygenic autoimmune diseases such as Sjögren's syndrome (SS), ulcerative colitis (UC), systemic lupus erythematosus (SLE), and dermatomyositis (DM) (61–64). Future research is required to determine whether, based on the shared pathologic features of these ILDs with APECED pneumonitis, these ILDs may also be responsive to the lymphocyte-directed therapy that is effective in APECED pneumonitis and GLILD. In addition, whether other primary immune dysregulatory disorders that manifest with ILD such as STAT3 gain-of-function (GOF), CTLA4 haploinsufficiency, and LRBA deficiency share common immunopathological mechanisms with APECED pneumonitis merits future investigation (65–68).

CONCLUSION

Herein, we highlighted clinical, radiographic, pulmonary function, autoantibody, immunological, and histological abnormalities of APECED pneumonitis, a previously-unrecognized manifestation of AIRE-deficiency that causes significant morbidity and mortality when untreated. Periodic screening with chest CT and bronchoscopic performance of endobronchial biopsies to reveal the characteristic compartmentalized immunopathology of pneumonitis have important implications for early diagnosis and initiation of lymphocyte-directed immunomodulation that can remit pneumonitis and prevent irreversible pulmonary complications. The common immunological and histological features between APECED pneumonitis and ILDs seen in secondary AIRE-deficiency states (thymoma, RAG deficiency), and certain polygenic autoimmune disorders (SS, UC, SLE, DM) suggest that the pathogenesis of autoimmune lung disease is shared among disorders of central immune tolerance and show promise for the potential efficacy of a similar lymphocyte-directed immunomodulatory regimen for these common ILDs.

AUTHOR CONTRIBUTIONS

EF conducted the literature review and wrote the initial draft of the manuscript. ML revised the manuscript. All authors contributed to the article and approved the submitted version.

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REVIEW
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B Cell Dysregulation in Common Variable Immunodeficiency Interstitial Lung Disease

Erik M. Matson, Miranda L. Abyazi, Kayla A. Bell, Kevin M. Hayes and Paul J. Maglione *

Pulmonary Center, Section of Pulmonary, Allergy, Sleep & Critical Care Medicine, Department of Medicine, Boston University School of Medicine, Boston Medical Center, Boston, MA, United States

Common variable immunodeficiency (CVID) is the most frequently diagnosed primary antibody deficiency. About half of CVID patients develop chronic non-infectious complications thought to be due to intrinsic immune dysregulation, including autoimmunity, gastrointestinal disease, and interstitial lung disease (ILD). Multiple studies have found ILD to be a significant cause of morbidity and mortality in CVID. Yet, the precise mechanisms underlying this complication in CVID are poorly understood. CVID ILD is marked by profound pulmonary infiltration of both T and B cells as well as granulomatous inflammation in many cases. B cell depletive therapy, whether done as a monotherapy or in combination with another immunosuppressive agent, has become a standard of therapy for CVID ILD. However, CVID is a heterogeneous disorder, as is its lung pathology, and the precise patients that would benefit from B cell depletive therapy, when it should administered, and how long it should be repeated all remain gaps in our knowledge. Moreover, some have ILD recurrence after B cell depletive therapy and the relative importance of B cell biology remains incompletely defined. Developmental and functional abnormalities of B cell compartments observed in CVID ILD and related conditions suggest that imbalance of B cell signaling networks may promote lung disease. Included within these potential mechanisms of disease is B cell activating factor (BAFF), a cytokine that is upregulated by the interferon gamma (IFN-γ):STAT1 signaling axis to potently influence B cell activation and survival. B cell responses to BAFF are shaped by the divergent effects and expression patterns of its three receptors: BAFF receptor (BAFF-R), transmembrane activator and CAML interactor (TACI), and B cell maturation antigen (BCMA). Moreover, soluble forms of BAFF-R, TACI, and BCMA exist and may further influence the pathogenesis of ILD. Continued efforts to understand how dysregulated B cell biology promotes ILD development and progression will help close the gap in our understanding of how to best diagnose, define, and manage ILD in CVID.

Keywords: common variable immune deficiency, CVID, GLILD, interstitial lung disease, TACI, BAFF-R, rituximab, B cell activating factor

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John R. Hurst, University College London, United Kingdom

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Javier Chinen, Baylor College of Medicine, United States

***Correspondence:**
Paul J. Maglione
pmaglion@bu.edu

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INTRODUCTION

Primary antibody deficiencies (PADs) are the most prevalent form of immunodeficiency and are defined by disruption of a patient's ability to generate functional antibodies. They are further classified by the mechanism of disruption and type of antibody affected. For example, X-linked agammaglobulinemia is an antibody deficiency defined by a reduction in all antibody classes due to a severe block in B cell differentiation, and hyper IgM syndrome is a deficiency characterized by defective B cell isotype class switching that results in lower levels of IgG and IgA, and higher IgM (1–3). The lack of a complete antibody arsenal typically predisposes PAD patients to recurrent bacterial and viral infections; however, the severity and prevalence of symptoms varies with type of PAD as well as individual manifestations of those with the same PAD.

The most prevalent symptomatic PAD is common variable immune deficiency (CVID) which is classified by profound reduction in IgG as well as IgA or IgM due to impaired B cell differentiation (4). Affecting 1:25,000 individuals, patients are typically diagnosed between the ages of 20 and 40 (5). Immunoglobulin replacement therapies can be used to limit infections, however about half of CVID patients develop non-infectious complications such as autoimmunity, lung and/or gastrointestinal disease, and malignancy despite this therapy (6). Moreover, these non-infectious complications occur in CVID more frequently than other forms of PAD for reasons that are poorly understood (7, 8). This suggests the presence of genetic, immunological, and/or environmental factors, and not simply antibody deficiency alone, drive the development of inflammatory complications in PAD. Yet, these complex etiologies remain poorly understood. Consequently, non-infectious complications are the leading cause of morbidity and mortality in CVID (9, 10).

The lung, as a mucosal surface regularly exposed to exogenous pathogens, is one of the organs most affected by the infectious and non-infectious complications of CVID. Upper respiratory tract infections by encapsulated bacteria are common in patients, leading to airway inflammation, impaired host defense, permanent tissue damage, and frequently bronchiectasis - an irreversible dilation of the bronchial airways (11). While bronchiectasis is likely the most common pulmonary complication of CVID, interstitial lung disease (ILD) also occurs in about 1 out of 3 CVID patients and accounts for a larger percentage of mortality (9, 10, 12). Radiological findings that distinguish CVID ILD typically include pulmonary nodules, ground glass opacities, and mediastinal lymphadenopathy (13). Additionally, biopsies typically reveal benign lymphoproliferation and granulomatous inflammation leading this form of interstitial lung disease to be labeled granulomatous-lymphocytic interstitial lung disease (GLILD) (1, 13). The exact cause of ILD in CVID remains unclear and does not require the presence of bronchiectasis or history of pneumonia, suggesting that infection is not an underlying cause in many cases (14). Immunoglobulin replacement therapy typically does not ameliorate the development of ILD in CVID, and current therapeutic approaches rely on immunomodulatory drugs (15). While treating ILD, these immunomodulatory drugs may also increase the risk of infection or malignancy in these patients already vulnerable for these complications, particularly because a therapeutic endpoint is often unclear (16). Greater understanding of ILD pathogenesis in CVID is needed to develop safer and more effective therapeutic approaches.

Perhaps a key to understanding ILD pathogenesis in CVID is the fact that it frequently occurs together with other non-infectious complications, like autoimmune cytopenia and splenomegaly, which are driven by mechanisms of immune dysregulation (17). Additionally, there are a number of monogenic antibody deficiency syndromes that present with ILD of a similar pathology to that seen in CVID patients (18). These include patients with gain-of-function mutations of *PI3KD* that develop the CVID-like activated PI3Kδ syndrome defined by lymphoid hyperplasia, which can affect the airways. Activated PI3Kδ syndrome can be ameliorated by rapamycin, which reduces resultant hyperactive mTOR signaling in lymphocytes, or targeted inhibition with the PI3Kδ inhibitor leniolisib (19, 20). Similarly, patients with genetic deficiency of cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) or a protein vital for its vesicular trafficking, lipopolysaccharide (LPS)-responsive and beige-like anchor protein (LRBA), develop inflammatory complications that are responsive to CTLA-4-Ig, known as abatacept (21, 22). These examples highlight the potential of precision immunomodulatory treatments for ILD as well as other non-infectious complications of CVID based upon identification of an underlying genetic lesion.

Despite CVID being defined by impaired antibody production, B cells appear to play an important role in ILD pathogenesis. Pulmonary B cell hyperplasia is a defining feature of CVID ILD, particularly in patients with biopsy proven follicular bronchiolitis, lymphocytic interstitial pneumonia, and nodular lymphoid hyperplasia of the lungs (23). Notably, ILD occurs far less commonly in X-linked agammaglobulinemia, a form of PAD where B cells are absent (7). Numerous studies have found B cell-depletive therapy with rituximab to be efficacious for CVID ILD (23–27). We conducted the largest study of rituximab monotherapy for CVID ILD, finding clear efficacy of this intervention over supportive care (28). ILD recurred after rituximab in about 1/3rd of subjects, but this recurrence could be limited by additional immunosuppression with azathioprine or mycophenolate. ILD recurrence was associated with increased levels of B cell activating factor (BAFF) in the blood and lungs, a key cytokine for B cell activation and survival (28). While these results do not prove that B cells are pathogenic in CVID ILD, they provide justification for deeper consideration and further research efforts to understand how these lymphocytes may contribute to disease. In the effort to summarize our understanding of how B

Abbreviations: APRIL, a proliferation-inducing ligand; BAFF, B cell activating factor; BAFF-R, BAFF receptor; BCMA, B cell maturation antigen; COPD, chronic obstructive pulmonary disease; CSR, class-switch recombination; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; CVID, common variable immunodeficiency; GLILD, granulomatous lymphocytic interstitial lung disease; GOLD, Global Initiative for Chronic Obstructive Lung Disease; IKKα, IκB kinase; iBALT, induced bronchus-associated lymphoid tissue; ILD, interstitial lung disease; LRBA, lipopolysaccharide (LPS)-responsive and beige-like anchor protein; NIK, NF-κB-inducing kinase; PAD, primary antibody deficiency; STAT1, signal transducer and activator of transcription 1; TACI, transmembrane activator and CAML interactor; TI, T cell-independent; TRAF3, TNF receptor-associated factor 3.

cells may contribute to CVID ILD, we will review mechanisms of B cell dysfunction described in CVID and non-CVID lung diseases alike. We apply particular focus upon BAFF-related B cell biology given the considerable research in CVID and other lung diseases that has been recently conducted.

It is important to note that not all ILD found in CVID may be the same. It has been suggested that there are diverse forms of ILD afflicting CVID patients (29). We have found evidence of B cell hyperplasia and heightened BAFF responses in CVID, specifically with biopsy-proven forms of benign lymphoproliferative interstitial lung disease. This is a spectrum of pulmonary pathology that starts with follicular bronchiolitis, when disease is limited to peribronchial areas, and progresses to lymphocytic interstitial pneumonia and nodular lymphoid hyperplasia, when inflammation becomes more diffuse within the lung parenchyma (30). CVID ILD can also manifest as other types of pathology, such as non-specific interstitial pneumonia, prominent granulomatous inflammation, or organizing pneumonia (12, 14). It may be important to confirm ILD by performing lymphocyte phenotyping of biopsies to gain a specific pathology diagnosis, like lymphocytic interstitial pneumonia, rather than label all forms of presumed ILD on CT scan as GLILD and treat them the same. It is likely that CVID ILD pathology with prominent B cell follicles, such as follicular bronchiolitis and lymphocytic interstitial pneumonia, may be more responsive to B cell-targeted therapy. Variability among CVID ILD pathology may mean that some cases are more responsive to BAFF or B cell-targeted therapy than others.

BIOLOGY OF BAFF AND ITS RECEPTORS

BAFF and a proliferation-inducing ligand (APRIL), are members of the tumor necrosis factor family of ligands that share receptors to promote activation and survival of B cells. BAFF and APRIL are elevated in the blood of CVID patients (31, 32). BAFF may contribute to lung disease in CVID as its levels were found to be highest in CVID patients with progressive ILD (28). APRIL levels were not found to be also elevated in this study. A variety of cell types are capable of producing BAFF in response to type I and type II interferons as well as pattern recognition receptor engagement, including dendritic cells, monocytes, and neutrophils (33). BAFF is expressed as a type II transmembrane protein that is processed at a furin cleavage site to release soluble BAFF (33, 34). Upon release from the cell membrane, BAFF can assemble into homotrimers or oligomeric, capsid-like 60-mers (35). Alternative splicing of BAFF generates a shorter isoform (ΔBAFF) that is co-expressed and associates with BAFF but interferes with proteolytic cleavage at the membrane (36). Thus, soluble BAFF can have distinct functional impact upon B cells depending on its abundance, multimeric state, and isoform.

The effects of BAFF are influenced by the specific receptor it binds. BAFF can signal *via* three receptors, BAFF receptor (BAFF-R), transmembrane activator and CAML interactor (TACI), and B cell maturation antigen (BCMA), while APRIL signals through TACI and BCMA only (**Table 1**) (37). BAFF receptors are differentially expressed across developmental subsets of B cells to regulate intracellular signaling pathways related to B cell activation, survival, and maturation (37–39). Expression of BAFF-R is absent on pre-B cells in the bone marrow until development into immature B cells, coinciding with establishment of BAFF-R as the predominant BAFF receptor in naive and transitional B cells (39). TACI expression increases with development into marginal zone and memory B cells as well antibody producing cells (38, 40). Expression of BCMA is mainly restricted to plasma cells (38, 41–43).

Along with differences in expression during B cell maturation, there are distinguishing features regarding BAFF-R signaling compared to other receptors for BAFF (**Figure 1**). In addition to activating the canonical NF-κB and phosphoinositide 3-kinase pathways, BAFF-R engagement of trimeric or oligomeric BAFF activates the non-canonical NF-κB pathway and upregulates expression of proteins in the Bcl-2 family that enhance B cell survival (44–46). Non-canonical NF-κB signaling requires activation of NF-κB-inducing kinase (NIK), a kinase that is

TABLE 1 | Important characteristics of the receptors for BAFF.

	BAFF-R (*TNFRSF13C*)	TACI (*TNFRSF13B*)	BCMA (*TNFRSF17*)
B cell subset expression	Naïve & transitional B cells	Marginal zone & class-switched memory B cells	Plasma cells
Ligands	BAFF trimer, BAFF 60mer	BAFF 60mer, APRIL, HSPGs	BAFF, APRIL
TRAF Interactions	TRAF3	TRAF2	TRAF1
	TRAF6	TRAF3	TRAF2
	TRAF2 (thru TRAF3)	TRAF5	TRAF3
		TRAF6	TRAF5
			TRAF6
Signaling pathways	Non-canonical NF-κB	Canonical NF-κB	Canonical NF-κB
	Canonical NF-κB	NFAT	
	PI3K-Akt	MyD88-dependent CSR	
Effects upon B cells	Pro-survival	Cell cycle arrest	Survival of plasma cells
	Enhanced proliferation	Apoptosis	
	Resistance to apoptosis	TI class switching to IgG, IgA	
		Plasma cell differentiation	
Extracellular CRDs	1 (shorter)	2	1
Soluble receptor processing	ADAM10, ADAM17 (BAFF & TACI-dependent)	ADAM10, γ-secretase, ADAM17	γ-secretase

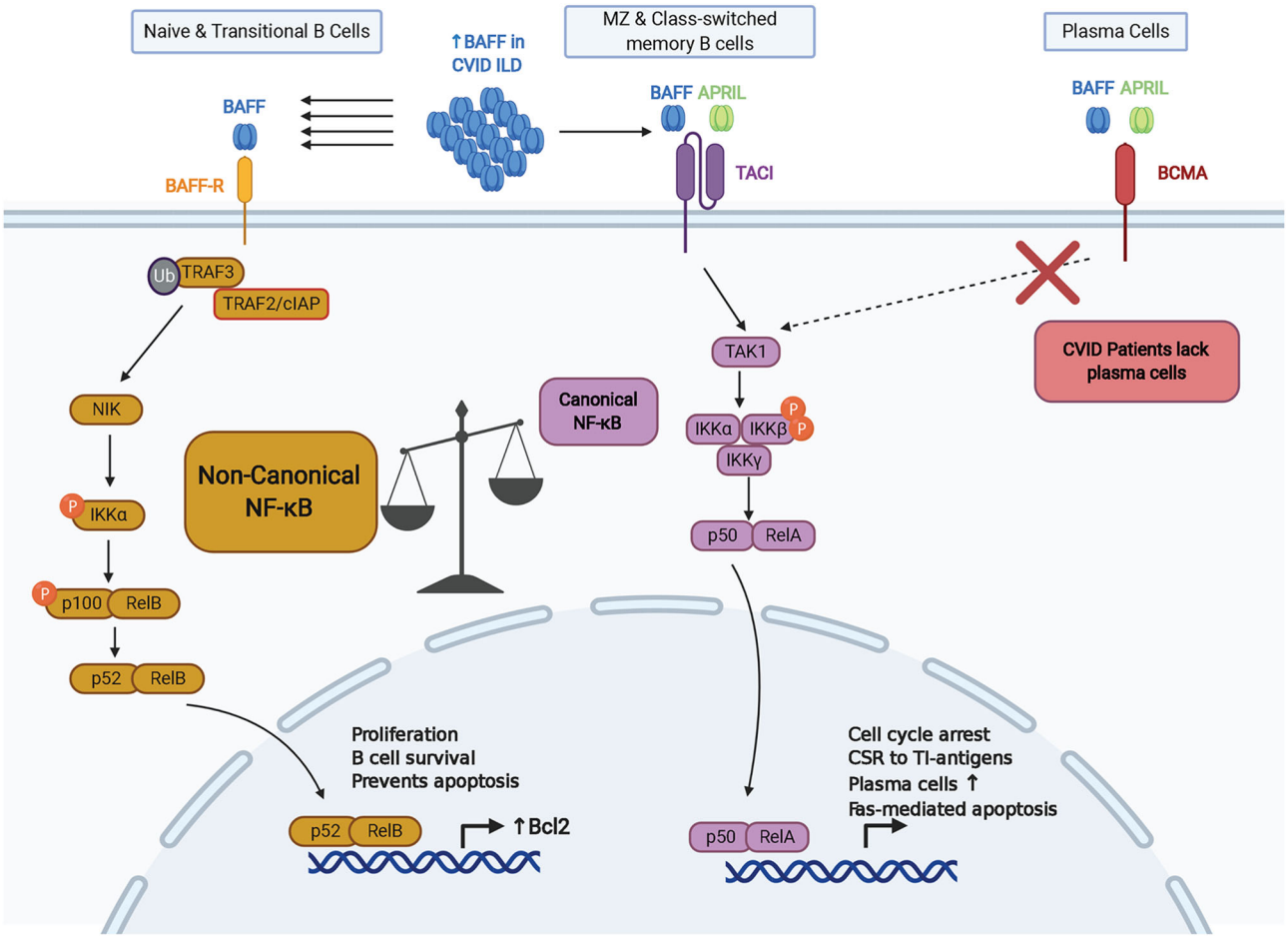

FIGURE 1 | Key aspects of BAFF-R, TACI, and BCMA signaling within the context of CVID. BAFF-R is distinguished by its ability to signal *via* the non-canonical NF-kB pathway to induce Bcl-2 and other pro-survival factors. Lack of memory B cells and plasma cells expressing TACI and BCMA in CVID may increase signaling *via* BAFF-R. CSR, class-switch recombination; TI, T-independent.

targeted for constitutive degradation while in complex with TNF receptor-associated factor 3 (TRAF3) in unstimulated B cells (47, 48). Ligation of BAFF to BAFF-R induces the targeted degradation of TRAF3, allowing NIK to accumulate and induce IκB kinase (IKKα)-dependent cleavage of p100 into p52 which associates with RelB to alter transcriptional activity (49–54). TRAF molecules such as TRAF2, TRAF3, TRAF5, and TRAF6, are recruited to the intracellular domain of BAFF receptors to mediate downstream signaling pathways in B cells through the canonical and non-canonical NF-κB pathways, AP-1 signaling, and MyD88-dependent class switch recombination in cooperation with TLRs (51, 55–58). B cell survival is also enhanced through the cooperation of BAFF-R with CD19 in regulating the activity of phosphoinositide 3-kinase (59). BAFF-R has greater affinity for BAFF compared to TACI and BCMA (60, 61). Due to its ability to promote survival through the non-canonical NF-κB and phosphoinositide 3-kinase pathways, expression from early stages of B cell maturation, and high affinity for BAFF, BAFF-R is positioned as a chief mediator of BAFF activity. Further efforts are needed to determine how significant the role of BAFF-R is in the pathogenesis of CVID-related complications.

Unlike BAFF-R, TACI signal activation requires binding to a higher-order oligomeric BAFF complex, such as the BAFF 60mer (62). BAFF signaling through TACI activates the canonical NF-κB pathway and upregulates expression of genes involved with cell cycle arrest, cell death, and class switch recombination (CSR) in response to T cell-independent (TI) antigens (45, 63, 64). In line with the role of TACI in TI responses, BAFF and APRIL induce IgG and IgA CSR *via* TACI through MyD88 (64). TACI interacts with mechanistic target of rapamycin (mTOR) *via* MyD88 to contribute to TACI-mediated NF-κB activation, association with TLRs, and IgG class switching in response to TI antigens (65). TACI also appears to have a regulatory role in antibody production from B cells stimulated with BAFF and CD40, which indicates a homeostatic role in regulating T cell-independent versus T cell-dependent antibody production (66). TACI can also signal through the nuclear factor of activated T cells (NFAT) pathway (67). Alternative splicing of TACI transcripts can generate a short isoform that induces strong

activation of the NF-κB pathway and has distinct localization within B cells compared to the full-length isoform (68, 69). Importantly, TACI signaling promotes expression of BLIMP-1, a transcription factor that induces cell cycle arrest and plasma cell differentiation by inhibiting expression of Bcl-6 and Pax5 (63, 70). Interestingly, Pax5 has been characterized as a lineage biomarker for a subset of rituximab-treated B cell lymphoma patients who relapse with CD20-negative B cells (71–74). However, the role of Pax5 in the development and progression of non-infectious complications in CVID remains to be characterized.

BAFF AND ITS RECEPTORS IN CVID

Germline mutations in *TNFRSF13B*, the gene that encodes TACI, are observed in 5-10% of CVID patients (75, 76). TACI-deficient patients are known to have an increased rate of autoimmunity and lymphoproliferative disease in CVID in association with increased autoreactive B cell selection and survival (77, 78). There may a greater risk of progressive ILD in CVID patients with certain TACI mutations compared to other CVID patients (28). The C104R and A181E variants are the most common variants in TACI that are considered likely pathogenic (**Figure 2**). The C104R mutation disrupts a disulfide bond in the extracellular cysteine rich domain 2 (CRD2) to diminish TACI ligand binding capacity and TACI-mediated activation of canonical NF-κB signaling (79). The A181E TACI variant affects the CAML binding site located in the transmembrane domain does not interfere with ligand binding or surface expression but fails to activate NF-κB signaling (79). Several other CVID-associated genetic variants of TACI have been identified in clinical settings and further characterization of these variants may provide insight into TACI's role in regulation of the BAFF/APRIL signaling axis in CVID and other diseases (75–77, 79–82). A global cohort analysis revealed that although mutations in *TNFRSF13B* are prevalent in CVID and healthy populations, there is an excess of rare derived alleles of *TNFRSF13B* in CVID cohorts compared to healthy individuals of the same population, indicating that defects in TACI are contributory toward manifestations of CVID (80). However, given the prevalence of the same variants in healthy populations, *TNFRSF13B* mutations are likely disease-modifying rather than disease-causing.

Regarding BAFF-R, a homozygous in-frame deletion that results in the loss of eight amino acids within the transmembrane region was identified in siblings with hypogammaglobulinemia (83). The two siblings had reduced serum IgG and IgM, but normal level of IgA. Class-switched memory B cells were lacking in these patients, and they did not have a medical history of autoimmune or lymphoproliferative complications. Also, a P21R variant of BAFF-R has been

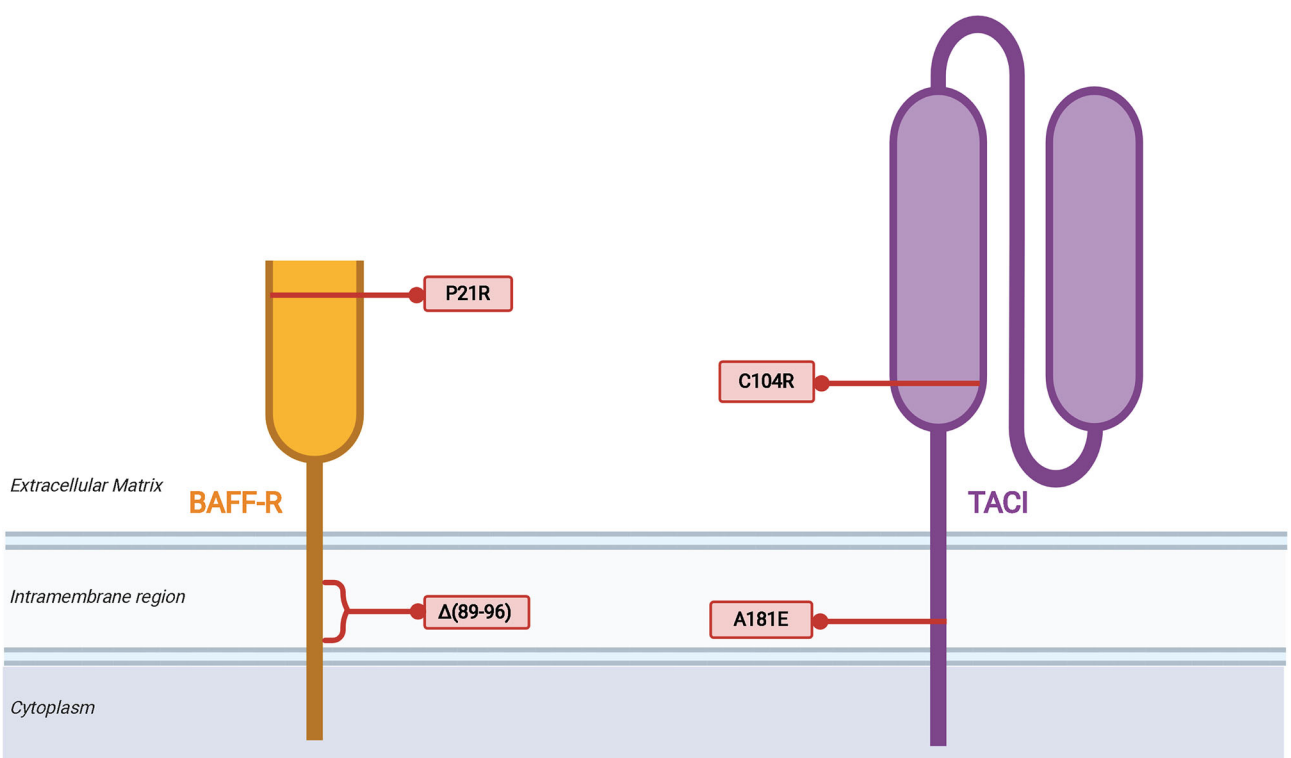

FIGURE 2 | Mutations of *TNFRSF13B* (TACI) associated with CVID. The variants listed are limited to the two most common, C104R and A181E, which are discussed in the text, as well as two other illustrative examples of how disruption of TACI can impair B cell function. Proposed mechanisms of biochemical disruption of certain variants included.

identified that interferes with BAFF-R complex formation, has reduced capacity to bind BAFF, and impairs BAFF-mediated NF-κB2 activation (84). B cells from patients with the BAFF-R P21R mutation lacked an increase in cell number and IgM secretion in response after stimulation with CpG DNA, anti-IgM, and BAFF. The BAFF-R P21R allele is found in 10.2% of CVID patients and 6.7% of healthy controls. Three additional heterozygous BAFF-R variants have been identified in a CVID cohort, all of which are present in healthy controls as well and their role in CVID remains to be defined (85).

SOLUBLE BAFF RECEPTORS

Each of the three BAFF receptors can be proteolytically processed to generate soluble molecules that function as decoy receptors in circulation (**Figure 3**). These soluble BAFF receptors add another layer to regulation of BAFF and APRIL-mediated homeostasis in B cells, prompting investigations into their utility in pharmacologic and diagnostic applications (86). Upon binding to BAFF, the extracellular domain of BAFF-R is processed by a metalloprotease (ADAM10) only in cells that also express TACI (87). This regulated processing is different from that of TACI and BCMA, receptors that undergo constitutive processing to release soluble fragments (88, 89). The BAFF trimer induces processing of BAFF-R by ADAM10, whereas TACI processing is unaffected by BAFF trimer stimulation (87). BAFF 60-mers are capable of stimulating processing of BAFF-R and TACI by both ADAM10 and ADAM17 (87). In the same study, the two metalloproteases, ADAM10 and ADAM17, demonstrated differential activity with respect to the activity state of B cells with increased ADAM10 activity on resting and TLR9-activated B cells, and ADAM17 processes BAFF-R on dark zone and germinal center B cells. Inhibition of ADAM10, responsible for processing of BAFF-R and TACI, was then shown to increase BAFF-dependent survival and secretion of IgM from B cells.

TACI is constitutively processed by ADAM10 on the surface of B cells to release the soluble extracellular domain of TACI capable of binding to BAFF and APRIL (88). Then γ-secretase, an intramembranous protease, cleaves the remaining membrane-proximal TACI fragment to prevent receptor-dependent activation of canonical NF-κB signaling (88). There is conflicting evidence supporting the capacity of the extracellular domain of TACI fused to an immunoglobulin Fc domain (TACI-Fc) to induce reverse signaling in macrophages through membrane bound BAFF

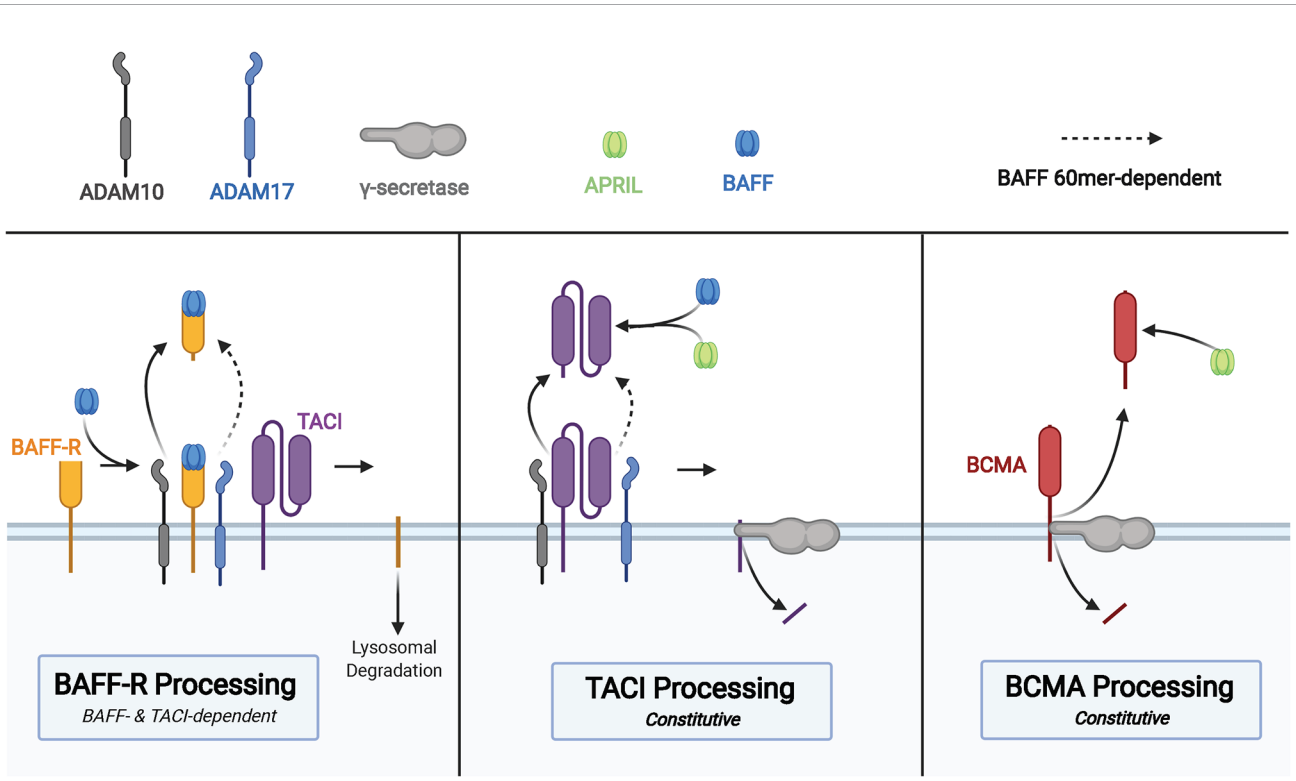

FIGURE 3 | Membrane processing of human BAFF receptors. Cleavage of the BAFF-R ectodomain is induced by BAFF binding in cells that co-express TACI. Processing of BAFF-R by ADAM10 is induced by binding to BAFF trimers and binding of BAFF 60mer to BAFF-R induces ADAM17 processing of BAFF-R. The membrane-bound C-terminal fragment of BAFF-R is degraded in lysosomes after cleavage of the ectodomain. TACI is cleaved in a constitutive manner by ADAM10, followed by cleavage of the membrane-bound C-terminal fragment by γ-secretase. sTACI exhibits homotypic assembly and binds to BAFF and APRIL to reduce NF-κB activation and B cell survival, with TACI-Fc demonstrating similar capabilities. BCMA is constitutively cleaved by γ-secretase to release sBCMA consisting of the ectodomain and a portion of the transmembrane domain of BCMA. sBCMA is a decoy for APRIL-induced NF-κB activation but does not block BAFF-mediated NF-κB activation. However, BCMA-Fc is capable of binding both APRIL and BAFF to block NF-κB activation.

and APRIL (90, 91). Studies that interrogate the role of soluble TACI must take into consideration differences in amino acid composition of endogenous sTACI compared to that of TACI-Fc due to demonstrated differences in BAFF/APRIL binding between sBCMA and BCMA-Fc (89). Subtle differences in the amino acid composition may have drastic effects on ligand binding capacity of the extracellular domain, as point mutations in TACI are capable of diminishing affinity for ligand, processing of TACI, and even processing of BAFF-R (87). Thus, the biological impact of soluble TACI remains incompletely understood.

BCMA is constitutively processed by γ-secretase, a process that acts to reduce surface BCMA and consequently regulate the number of plasma cells in the bone marrow, given the importance of this receptor for plasma cell survival (89). Although BCMA is able to bind BAFF and APRIL to induce canonical NF-κB signaling, soluble BCMA (sBCMA) is able to bind APRIL but does not block BAFF-mediated activation of NF-κB in HEK cells transfected with BCMA (89). The same study also found recombinant BCMA-Fc to bind BAFF and APRIL, leading to inhibition of BAFF and APRIL-mediated NF-κB signaling through BCMA. Quantification of serum BCMA revealed markedly reduced levels among patients with severe PAD, such as CVID and XLA (92). Evaluation of immunoglobulin deficiencies in CVID and other PADs often requires repeated vaccine challenges and discontinuation of immunoglobulin replacement therapy, which increase patient susceptibility to infection and may take several weeks (93–95). Methods of diagnosing PAD requiring immunoglobulin replacement that reduces diagnostic delay and does not require treatment discontinuation, such as is the case with sBCMA measurement, could significantly improve clinical care and quality of life in those with PAD.

THE POTENTIAL CONTRIBUTION OF BAFF TO CVID ILD

CVID patients can have a significant increase in serum IgM corresponding to progression of ILD as determined by pulmonary function decline (96). This serum IgM increase is associated with hyperplasia of ectopic pulmonary B cells expressing IgM (28). B cell depletion with rituximab ameliorates CVID ILD, corresponding with improved pulmonary function and reduction of serum IgM, compared to those receiving supportive care (28). Moreover, the ILD recurrence that occurred in 1/3rd of study subjects within 2 years of receiving rituximab was also associated with serum IgM elevation (28). Thus, the presence and reemergence of B cells, corresponding with rising levels of serum IgM, may be quite fundamental to CVID ILD pathogenesis.

CVID patients who experienced ILD progression after rituximab had significantly elevated levels of BAFF in blood and lung tissue compared to CVID patients with stable ILD, no ILD, and healthy controls (28). IFN-γ upregulates signal transducer and activator of transcription 1 (STAT1) expression to act as a potent stimulus of BAFF production (97). Numerous reports that have found elevation of IL-12, IFN-γ, and related T helper type 1 cytokines in CVID patients with inflammatory complications (28, 98–105). Furthermore, plasma IFN-γ levels and STAT1 expression were elevated in CVID patients with progressive ILD and correlated with BAFF expression, and $CD14^+$ monocytes were identified as a prominent source of IFN-γ-induced BAFF production and STAT1 expression in CVID patients with progressive ILD (28). Together, these results implicate an IFN-γ:STAT1:BAFF axis in pathogenesis of ILD in CVID. Efforts to unravel fundamental biology and clinical importance of this IFN-γ and BAFF relationship in CVID are underway.

Heterozygous mutations of TACI found in CVID appear to be key for the persistence of autoreactive B cells through interaction with toll-like receptor (TLR) 7 and TLR9 (106). Moreover, when BAFF is elevated in non-CVID patients it has been shown that autoantigen-engaged B cells demonstrate enhanced survival and migration to follicular zone and marginal zone niches where they would normally be excluded (107, 108). While the relationship between B cell autoreactivity and ILD is unclear in CVID, it is possible that enhanced BAFF-R signaling in the absence of counterbalancing signals from TACI promotes pathogenic pulmonary B cell hyperplasia. Indeed, 3 patients with TACI mutations in our study of CVID all had progressive ILD that recurred after rituximab (28). Thus, in addition to the greater prevalence of progressive ILD in CVID patients with TACI mutations there was apparently greater resistance to B cell depletive therapy, possibly due to elevated signaling through BAFF-R.

BAFF-R is the predominant BAFF receptor expressed by the IgD+ B cells that make up the ectopic pulmonary follicles observed in CVID ILD, while TACI is expressed in the extrafollicular areas of the lung harboring plasmablasts expressing IgM and the proliferation marker Ki67 (28). BAFF-R is the principal BAFF receptor on B cells in CVID patients with autoimmune and lymphoid hyperplasia due to the lack of marginal zone, memory, and plasma cells in these patients that would otherwise express TACI and/or BCMA (109, 110). Elevated levels of BAFF enhance BAFF-R-mediated activation of the non-canonical NF-κB pathway to upregulate Bcl-2 survival signals and impair B cell apoptosis (45, 48). The expanded subset of naïve B cells in CVID ILD were observed to induce expression of Bcl-2 and RelB to a level that is significantly greater in CVID patients with progressive ILD compared to healthy controls (28). Enhanced activity of BAFF-R signaling in response to elevated BAFF not only drives proliferation and resistance to apoptosis in naïve B cells, but may concurrently impair B cell maturation by drowning out BAFF-mediated maturation signals from TACI (45, 63). Excessive BAFF inhibits autophagy in B cells and reduces autophagosome marker LC3-II through mechanisms that depend on active Akt/mTOR signaling, suggesting that elevated BAFF can drive B cell survival through multiple mechanisms (111).

The extent of B cell contributions to pathogenesis of CVID ILD remains to be sufficiently defined. Like B cells, T cells are a prominent feature of CVID ILD pathology, and treatment with

azathioprine or mycophenolate mofetil in combination with rituximab improved clinical chest radiography scores and components of pulmonary function testing in patients with CVID ILD (15). A considerable portion of patients in this study relapsed after receiving this immunosuppressive combination in association with elevated B cells and activated $CD4^+$ T cells. Variations in the extent of immune cell compartment imbalance in CVID may enhance the progression of ILD due to mechanisms that remain unclear. T cells from CVID patients demonstrate increased frequencies of activated, memory, and effector populations with a lack of naïve and regulatory T cell subsets (112). The enhanced state of T cell activation and effector function in CVID may further contribute to the B cell hyperplasia observed in CVID ILD due to a lack of T cell-mediated regulation of B cell activity in addition to upregulation of non-canonical NF-κB signaling in B cells as a result of more widespread stimulation of CD40 through CD40L expressed on activated T cells (113, 114). The notable variability of clinical manifestations and aberrant immune cell compartments in CVID suggests that multiple aspects of immune system dysregulation may contribute to CVID ILD. Furthermore, efficacy of therapeutic depletion of B cells may stem from indirect effects upon leukocytes, such as T cells, that closely interact with B cells in CVID ILD.

Studies of lung disease with pathologic similarities to that observed in CVID ILD may also prove to be informative. For example, lymphocytic interstitial pneumonia makes up 15% of interstitial lung disease affecting Sjogren's syndrome patients (115). Similar to CVID, B cells appear to play a central role in the development of ILD in Sjogren's syndrome. Specifically, elevated levels of BAFF can be found in the serum, saliva, and salivary glands of Sjogren's syndrome patients in comparison to healthy controls (116–118). BAFF levels in these patients are also positively associated with the presence of autoantibodies, including anti-SSA and anti-SSB (119). Also, like we found in CVID ILD, elevated levels of BAFF seen in Sjogren's is associated with heightened interferon signaling through the JAK/STAT pathway in monocytes (28, 120). Elevated levels of BAFF in Sjogren's syndrome ultimately enables prolonged survival of B cells, which have been shown to aggregate into inducible bronchus-associated lymphoid tissue structures with pulmonary B cell follicles as in CVID ILD (121). A double-blind, randomized, placebo-controlled, multi-center, multi-national clinical trial (NCT02631538) that investigated the effects of rituximab and belimumab administration in 86 pSS patients was recently completed in June 2020. This trial contained four groups, including a placebo group, a group that received only belimumab, a group that received only rituximab, and a group that received both belimumab and rituximab. Results from this study have not been published yet, but they will put the implication of BAFF and aberrant B cell survival and signaling found in Sjogren's syndrome patients to the test.

Another chronic lung disease where there is increasing evidence for a role of B cells and BAFF is chronic obstructive pulmonary disease (COPD). Although COPD is commonly associated with smoking, anywhere between 25 and 45% of COPD patients have never smoked, suggesting that other factors contribute to the pathogenesis of this lung disease (122). The implication of the adaptive immune system in the development and progression of COPD becomes evident when considering the fact that there is a significantly greater number of B cells and CD4+ and CD8+ T cells in the airways and parenchyma of the lungs of COPD patients (123, 124). These excess B and T cells arise from induced bronchus-associated lymphoid tissue (iBALT) and form pulmonary follicles containing germinal center B cells and follicular T cells (123). Moreover, significantly more lymphoid follicles were found in the lungs of those who were diagnosed with COPD in comparison to smokers without COPD (124). Also, when categorizing COPD patients on the Global Initiative for Chronic Obstructive Lung Disease (GOLD) scale, a significant increase in the number and size of lymphoid follicles was seen in later-stage COPD patients in comparison to those in the earlier stages (124). The same study also performed immunofluorescence on lung samples and found the size of the lymphoid follicles identified in each of the aforementioned groups to be directly correlated to the percentage of BAFF-positive B cells, which co-localized with BAFF-R (124, 125). BAFF expression was also found to be elevated in the blood of COPD patients in comparison to non-smoking and smoking control subjects (124). Healthy, smoking controls and the early-stage COPD subjects, on the other hand, had a higher proportion of caspase-3-positive B cells, indicating apoptosis, in their pulmonary follicles in comparison to later-stage COPD subjects. These findings implicate dysregulation of the BAFF : BAFF-R axis in the progression of COPD, with the anti-apoptotic signals of BAFF-R promoting the B cell follicles that are a major component of pulmonary pathology, similar to what was found in CVID ILD.

CONCLUSION

There is increasing evidence that dysregulated B cell responses, such as those exacerbated by BAFF, promote the progression of ILD in CVID. This is supported by the adoption of B cell depletive therapy, either alone or in combination with other immunosuppression, as a fundamental component of CVID ILD treatment. Continued suppression of B cell activation through administration of immunosuppressive antimetabolite agents such as azathioprine or mycophenate, or potentially through inhibition of BAFF may help maintain CVID ILD in remission. B cell hyperplasia is a defining aspect of CVID ILD and is perpetuated *via* survival signals mediated by BAFF through BAFF-R. In addition to B cells, CVID ILD consists of prominent T cell infiltration which appears to also improve with B cell depletive therapy (23, 28). The link between B cells and T cells in the CVID lungs remains undefined, and whether depletion of B cells removes a vital antigen-presenting cell, lymphoid structure, source of chemokines, and/or another component required for T cell recruitment and persistence in the lungs is unknown. Further research is necessary to prove whether B cells fundamentally contribute to pathogenesis of CVID ILD, define the best way to achieve safe long-lasting suppression of dysregulated B cell responses, and accurately identify the individual CVID patients who would most benefit from B cell-targeted therapy. Moreover, we must elucidate

mechanisms by which the IFN-γ/STAT1/BAFF axis is elevated in CVID and other disorders. Further efforts to unravel the mechanisms by which BAFF and B cells become dysregulated in CVID offer potential to address these knowledge gaps in CVID and other forms of autoimmune and inflammatory disease.

AUTHOR CONTRIBUTIONS

EM, MA, KB, and KH drafted the manuscript. PM provided guidance and revisions. All authors contributed to the article and approved the submitted version.

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Bronchoalveolar Lavage Fluid Reflects a T_H1-CD21^{low} B-Cell Interaction in CVID-Related Interstitial Lung Disease

David Friedmann[1,2,3†], Susanne Unger[1,2,4†], Baerbel Keller[1,2], Mirzokhid Rakhmanov[1,2,5], Sigune Goldacker[1,2], Gernot Zissel[6], Björn C. Frye[6], Jonas C. Schupp[6,7], Antje Prasse[8,9] and Klaus Warnatz[1,2]*

[1] Divison of Immunodeficiency, Department of Rheumatology and Clinical Immunology, Medical Center—University of Freiburg, Faculty of Medicine, University of Freiburg, Freiburg, Germany, [2] Center for Chronic Immunodeficiency (CCI), Medical Center—University of Freiburg, Faculty of Medicine, University of Freiburg, Freiburg, Germany, [3] Faculty of Biology, University of Freiburg, Freiburg, Germany, [4] Institute of Experimental Immunology, University of Zurich, Zurich, Switzerland, [5] Center for Human Genetics and Laboratory Diagnostics (AHC), Martinsried, Germany, [6] Department of Pneumology, University Medical Center—University of Freiburg, Faculty of Medicine, University of Freiburg, Freiburg, Germany, [7] Pulmonary, Critical Care and Sleep Medicine, Yale University School of Medicine, New Haven, CT, United States, [8] Department of Respiratory Medicine, Hannover Medical School and Biomedical Research in End-stage and Obstructive Lung Disease Hannover, German Lung Research Center (DZL), Hannover, Germany, [9] Fraunhofer Institute for Toxicology and Experimental Medicine, Hannover, Germany

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Menno C. van Zelm, Monash University, Australia

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Julian Joseph Bosco, The Alfred Hospital, Australia

***Correspondence:**
Klaus Warnatz
Klaus.warnatz@uniklinik-freiburg.de

†These authors have contributed equally to this work

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Background: About 20% of patients with common variable immunodeficiency (CVID) suffer from interstitial lung disease (ILD) as part of a systemic immune dysregulation. Current understanding suggests a role of B cells in the pathogenesis based on histology and increased levels of BAFF and IgM associated with active disease corroborated by several reports which demonstrate the successful use of rituximab in CVID-ILD. It is debated whether histological confirmation by biopsy or even video-assisted thoracoscopy is required and currently not investigated whether less invasive methods like a bronchoalveolar lavage (BAL) might provide an informative diagnostic tool.

Objective: To gain insight into potential immune mechanisms underlying granulomatous and lymphocytic interstitial lung disease (GLILD) and to define biomarkers for progressive ILD by characterizing the phenotype of B- and T-cell populations and cytokine profiles in BAL fluid (BALF) of CVID-ILD compared to sarcoidosis patients and healthy donors (HD).

Methods: Sixty-four CVID, six sarcoidosis, and 25 HD BALF samples were analyzed by flow cytometric profiling of B- and T-cells and for cytokines by ELISA and Multiplexing LASER Bead technology.

Results: Both sarcoidosis and CVID-ILD are characterized by a predominantly T-cell mediated lymphocytosis in the BALF. There is an increase in T follicular helper (T_{FH})-like memory and decrease of regulatory T cells in CVID-ILD BALF. This T_{FH}-like cell subset is clearly skewed toward T_H1 cells in CVID-ILD. In contrast to sarcoidosis, CVID-ILD BALF contains a higher percentage of B cells comprising mostly CD21^{low} B cells, but less class-

switched memory B cells. BALF analysis showed increased levels of APRIL, CXCL10, and IL-17.

Conclusion: Unlike in sarcoidosis, B cells are expanded in BALF of CVID-ILD patients. This is associated with an expansion of T_{FH}- and T_{PH}-like cells and an increase in APRIL potentially supporting B-cell survival and differentiation and proinflammatory cytokines reflecting not only the previously described T_H1 profile seen in CVID patients with secondary immune dysregulation. Thus, the analysis of BALF might be of diagnostic value not only in the diagnosis of CVID-ILD, but also in the evaluation of the activity of the disease and in determining potential treatment targets confirming the prominent role of B-cell targeted strategies.

Keywords: common variable immunodeficiency, interstitial lung disease, cytokines, CD21low B cells, T_{FH} and T_{PH} cells

INTRODUCTION

Common variable immunodeficiency (CVID) is an antibody deficiency syndrome (www.esid.org) with a heterogeneous, mostly unknown pathogenesis. This most common primary immunodeficiency is defined by reduction of serum IgG, IgA, and/or IgM and impaired antibody responses together with disturbed memory B cell and plasma cell development (1, 2). Mutations in several genes have been associated with the clinical presentation of CVID, currently explaining only less than 20% of CVID cases (3, 4). Clinically, most CVID patients suffer from recurrent bacterial infectious diseases, particularly of the respiratory tract. This is frequently associated with the development of bronchiectasis over time (5). Additionally, around 50% of CVID patients have secondary noninfectious lymphoproliferative, autoimmune and inflammatory complications like autoimmune cytopenias, granulomatous disease, splenomegaly and lymphadenopathy, interstitial lung disease, enteropathy and hepatopathy (6) often contributing to a significantly reduced quality of life and increased morbidity and mortality (7–10).

Interstitial lung disease (CVID-ILD) is one of the main complications in CVID. It manifests in about 20% of CVID patients and may be present already at the initial diagnosis in a relevant subgroup of patients frequently leading to the misdiagnosis of sarcoidosis (11, 12). No infectious agent has been reliably identified as a trigger of the disease and CVID-ILD is felt to be part of the systemic lymphoproliferative immune dysregulation. It manifests variably with follicular bronchiolitis, lymphocytic interstitial pneumonia and nodular mostly granulomatous lung disease (13–15). Maglione et al. described B cell containing tertiary lymphoid germinal center (GC)-like structures within the affected lung tissue (16). Recently, they suggested that active CVID-ILD is driven by pulmonary B cell hyperplasia which is reflected by elevated BAFF-mediated apoptosis resistance and an increase in serum IgM (17). The pivotal role of B cells in the lung pathology is underpinned by the positive effect of B-cell depleting therapies on CVID-ILD (18).

The optimal form of treatment has however not yet been defined. IgG replacement therapy alone rarely prevents or improves CVID-ILD (15, 19, 20), thus immunosuppressive therapy is frequently used to control the pulmonary manifestations of the immune dysregulation (21).

Diagnosis is currently often based on CT morphology and pulmonary function tests (22, 23) with no additional histological or other confirmation. The need for confirmation by video-assisted thoracoscopic surgery (VATS) assisted lung biopsies is postulated by some (13), but not endorsed by others due to the invasive character of the procedure and the lack of significant impact on diagnosis in the majority of cases (24).

Therefore, we set out to retrospectively analyze the data of bronchoalveolar lavage (BAL) in patients with CVID as a less invasive procedure. The patients were seen at the Center for Chronic Immunodeficiency (CCI) in the years between 2004 and 2020.

METHODS

Patients and BAL Samples Processing

All patients fulfilled the criteria for CVID according to the European Society for Immunodeficiencies (ESID) (www.esid.org) and suffered from interstitial lung disease as determined by radiological and/or lung function abnormalities. The following clinical data was recorded (**Supplementary Table 1**): splenomegaly (defined as a diameter of greater than 11x4.7 cm proven by ultrasound or computer tomography (CT scan); generalized lymphadenopathy (LNs >1 cm in diameter in at least two different anatomical sites detected by clinical examination, ultrasound, or CT); autoimmune cytopenias (autoimmune hemolytic anemia or immune thrombocytopenia); enteropathy (based on clinical presentation, endoscopic analysis and histology when available), liver disease (based on clinical parameters, ultrasound, serum parameters and histology when available). In addition, all patients were classified according to EUROclass classification (25), considering the reduction of switched memory B cells (smB) and the expansion of $CD21^{low}$ B cells.

All procedures performed in this study were in accordance with the ethical standards of the institutional (FR 189/

12_120543) research committee and with the 1964 Helsinki declaration and its later amendments. Informed consent was obtained from all individual participants before inclusion into the study. Patients underwent bronchoscopy as part of clinical work-up, i.e. differential diagnosis of respiratory complaints and/or radiological abnormalities. BAL samples were obtained from 64 CVID patients (33 female and 31 male patients, age 17–73 years), 6 sarcoidosis patients (one female and five male patients, age 29 to 76 years and 25 healthy adult volunteers (12 female and 13 male, age 19–67 years). Five former smokers and three smokers could be identified (see **Supplementary Table 1**). BAL samples of diagnostic bronchoscopy were analyzed by the routine laboratory for overall cell counts, vitality, lymphocytes, T cells (including CD4 and CD8 T cell subsets), macrophages, neutrophils, eosinophils and basophils/mast cells as described by Frye et al (26). and the guidelines of the European Respiratory Society (27). Additional phenotyping of T and B cell subsets and cytokine production was performed *via* our research laboratory. Due to the retrospective character, not all investigations were performed from the same samples.

Immunophenotyping by Using Flow Cytometry

Cells from bronchoalveolar lavage were washed in Iscove's Modified Dulbecco's Medium (IMDM) or Roswell Park Memorial Institute (RPMI) media with 10% FCS and further processed for flow cytometry.

B-cell populations were characterized by staining for IgD, IgA, IgM, IgG, CD19, CD21, CD27 and CD38 expression and T cell subsets by their expression of CD3, CD4, CD8a, CD25, CD27, CD28, CD45, CD45RA, CCR6, CXCR3, CXCR5, PD-1, FoxP3, CTLA-4.

All applied antibodies and their vendors are listed in **Supplementary Table 2** in the Online Repository.

Data acquisition was performed on a Gallios flow-cytometer (Beckman Coulter, Miami, FL) or LSR Fortessa (BD Biosciences, Franklin Lakes, NJ). Data were analyzed using FlowJo software (Treestar, Ashland, OR).

Cytokine Levels in BALF

IL-4, IL-10, IL-12, IL-17, and CXCL10 (IP10) in BALF were analyzed by multiplex bead technology assays using the Luminex® xMAP® platform performed by Eve Technologies Corporation, Calgary, Alberta, Canada.

APRIL, BAFF, CXCL9, CXCL13, CXCL14, and CXCL10 in cell-free BALF were quantified using DuoSet ELISA Kits (R&D Systems) according to the manufacturer's protocol. All samples were measured in duplicates.

Statistical Analysis

Values were expressed as means ± SDs. Statistical significance was assessed by the unpaired T test for datasets with Gaussian distribution, or by the Mann-Whitney test for datasets without Gaussian distribution. The Kruskal-Wallis test or ordinary one-way ANOVA were used for multiple comparisons. Correlation data was assessed by simple correlation test.

Results were analyzed with the help of GraphPad Prism software (version 8.4.2; GraphPad Software, La Jolla, Calif), and p values of less than 0.05 were considered significant.

RESULTS

Lymphocytic Bronchoalveolar Lavage Fluid in the Majority of CVID-ILD

The routine diagnostic workup of the BAL samples revealed an increased total cell count. Absolute leukocyte counts were increased in 79% of CVID patients above normal range. These were significantly higher (22.0×10^6/100 ml +/− 14.5×10^6/100 ml) than in the control group with sarcoidosis (10.6×10^6/100 ml +/− 4.7×10^6/100 ml) (**Figure 1A**). In 83% of the CVID patients the analysis revealed an expansion of lymphocytes, 65% of the BALF were characterized by a relative increase in neutrophils and 37% of eosinophils (**Figure 1A**). In 59% of CVID patients, increased neutrophils were associated with the detection of concurrent bacterial or fungal infection. The slight increase in eosinophils could not be attributed to a specific cause and was similarly seen in sarcoidosis. Interestingly, nearly all of the genetically defined immunodeficiencies had no detectable eosinophils. Overall, the cellular composition of the main leukocyte cell differentiation lineages in BALF of CVID-ILD was not significantly different to sarcoidosis.

Also, similar to sarcoidosis, $CD3^+$ T cells were increased compared to the normal range in over 90% of CVID patients (**Figure 1B**), but in 67% of CVID patients there was an additional increase of B cells not seen in sarcoidosis (**Figure 1C**). The typically increased CD4/CD8 ratio in sarcoidosis was less frequently seen in CVID patients (**Figure 1D**).

Expansion of T_{FH} and T_{PH} Cells in BALF of CVID-ILD

Further CD8 T cell phenotyping revealed a similar distribution of effector memory subsets according to their CD27 and CD28 expression compared to patients with sarcoidosis (data not shown). In contrast, additional phenotyping of $CD4^+CD45RA^-$ memory T cells demonstrated an expansion of CXCR5-expressing T follicular helper (T_{FH})-like cells (**Figure 2A**) with a significant increase of CXCR3-expressing $T_{FH}1$-like cells and a decrease of CCR6-expressing $T_{FH}17$-like cells when compared to sarcoidosis (**Figure 2A**). Moreover, there was a significant increase of the recently described $CXCR5^{neg}PD1^{high}$ T peripheral helper (T_{PH})-like cell population (28) in BAL samples of CVID patients compared to patients with sarcoidosis (**Figure 2B**).

These changes were associated with a significant decrease of $FoxP3^+CD25^+$ T regulatory cells (Treg) among memory CD4 T cells (**Figure 2C**), expressing lower amounts of CD25 on their surface compared to sarcoidosis patients (**Figure 2D**). As a consequence, the ratio of $CXCR5^+$ T_{FH}-like cells to Tregs was significantly increased in CVID patients (**Figure 2E**).

We did not detect significant differences in regard to other T-cell populations (data not shown).

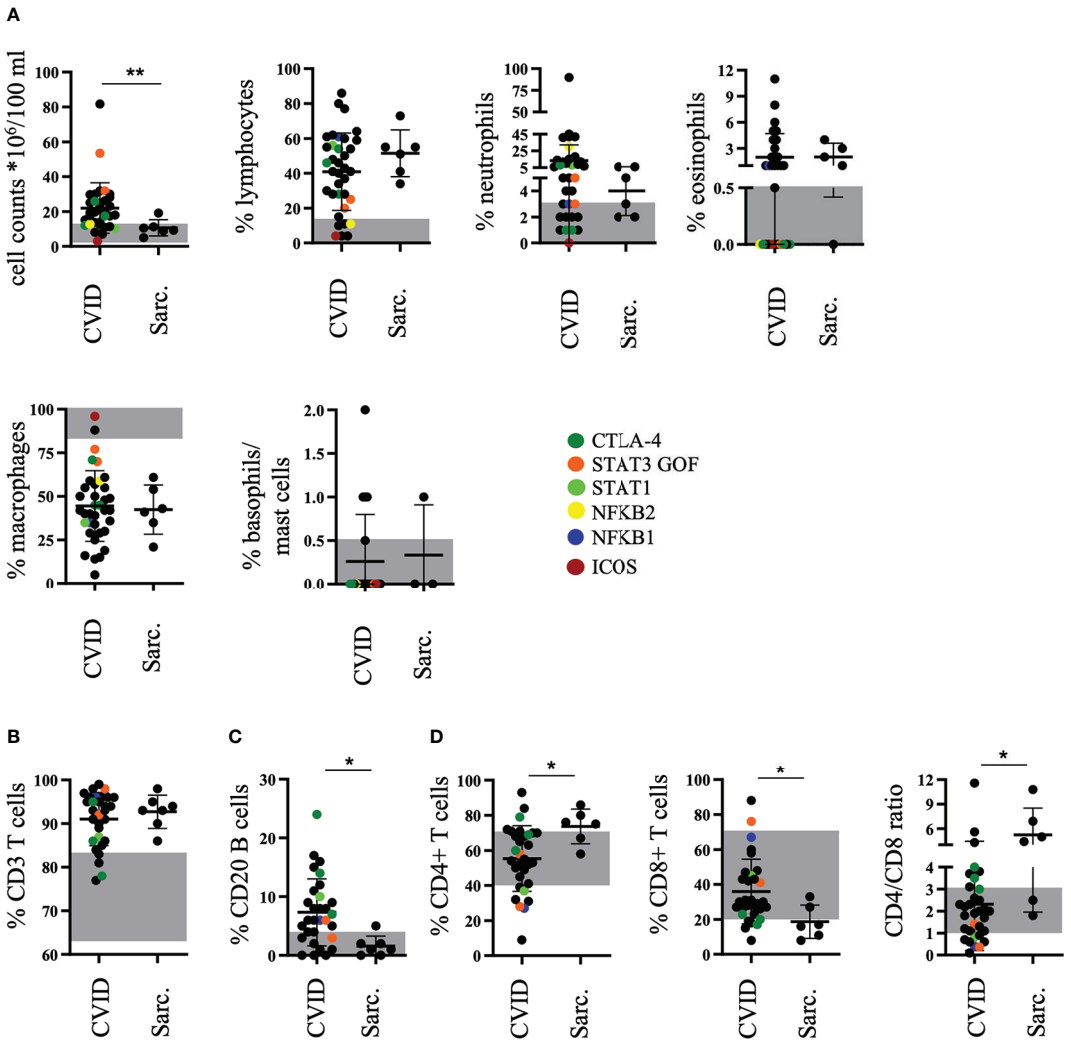

FIGURE 1 | Increased percentage of B cells in bronchoalveolar lavage fluid (BALF) of common variable immunodeficiency (CVID)-interstitial lung disease (ILD) compared to sarcoidosis. The diagnostic workup of the BALF of patients with CVID or sarcoidosis for cell counts, percentages of lymphocytes, neutrophils, eosinophils, macrophages, and basophils/mast cells **(A)**, CD3+ T cells **(B)**, CD20+ B cells **(C)**, as well as CD4+ and CD8+ T cells including CD4/CD8 ratio **(D)**. The normal range is marked in grey for each population and defined genetic defects are marked by color coding. Sarc., sarcoidosis. *P <.05, **P <.01.

The Expanded B-cell Population Consists Mainly of CD21^{low} B Cells in BALF of CVID-ILD

Since B cells are expanded in BALF of the majority of CVID patients we investigated their phenotype more closely (**Figure 3A**). As previously reported by our group (29) the main B cell population in the BALF of CVID patients with ILD were CD21^{low} B cells representing T-bet^{hi} B cells (30, 31) (**Figure 3B**). This population was significantly expanded compared to sarcoidosis, while plasmablasts were reduced in the CVID cohort (**Figure 3B**).

The majority of CD21^{low} B cells represented phenotypically as naïve-like CD27^{neg}IgD^{pos}IgM^{pos} and atypical CD27^{neg}IgD^{neg}IgM^{pos} B cells (**Figure 3B**). CVID patients differed significantly from sarcoidosis patients in regard to the expansion of their naïve-like B cells within the CD21^{low} compartment as well as the reduction of atypical and switched memory B cells within the CD21^{pos} compartment (**Figure 3B**).

As expected from blood data within the CD27^{pos} memory compartment, CVID patients showed a relative reduction of IgA^{pos} switched memory B cells and increase of IgM-only cells both among CD21^{low} and CD21^{pos} B cells compared to sarcoidosis (**Figure 3B**). Interestingly, especially CD27^{pos} CD21^{low} B cells comprise a comparable amount of IgG^{pos} B cells in the BALF compared to sarcoidosis patients while these cells are usually reduced in peripheral blood of CVID patients (25).

Increased APRIL, IP10, and IL-17 Concentrations in BALF of CVID-ILD

ELISAs of BAL fluids of 30 CVID patients and 25 healthy donors revealed an increased concentration of APRIL in BALF of CVID

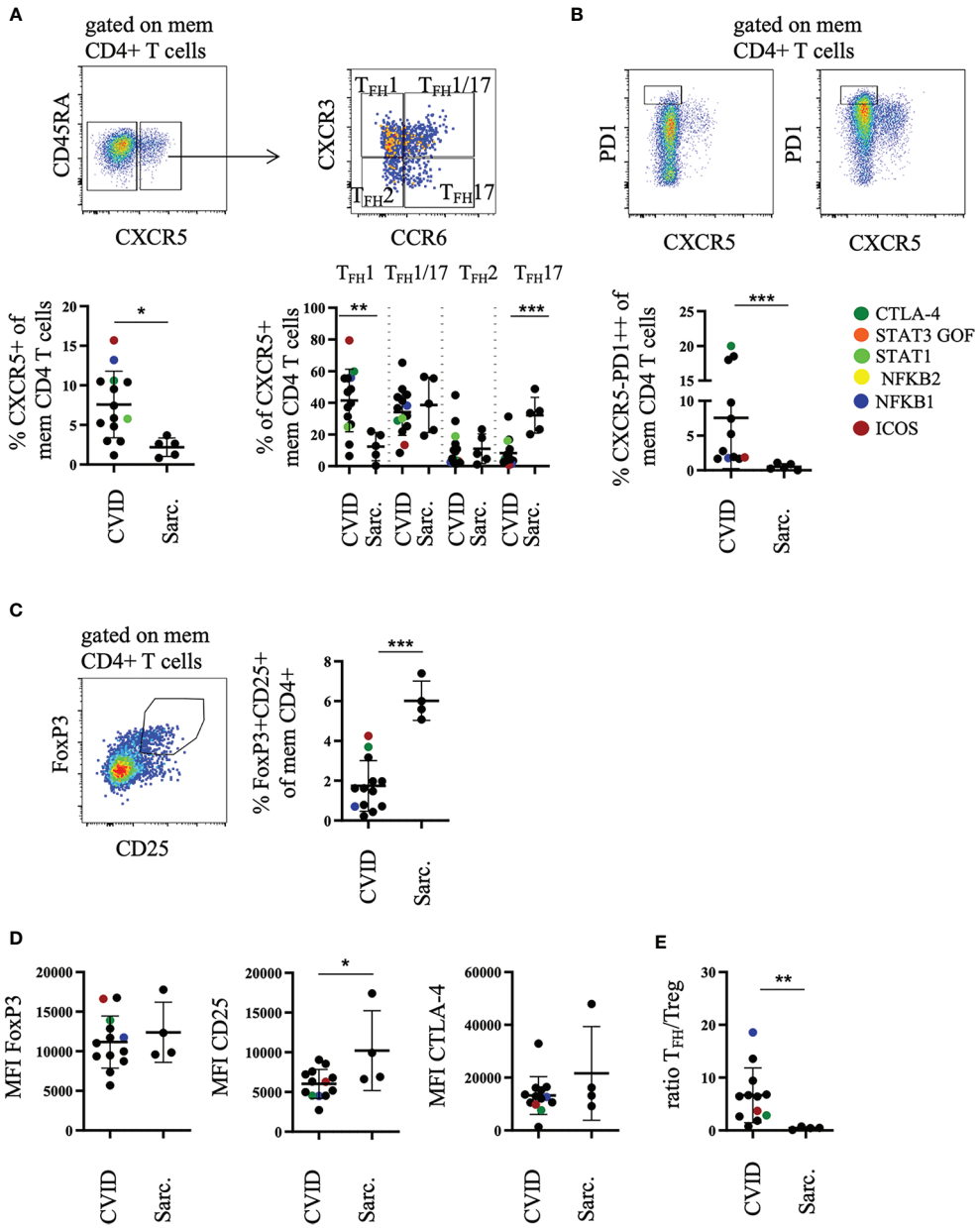

FIGURE 2 | Increased percentage of $T_{FH}1$-like and T_{PH} cells in bronchoalveolar lavage fluid (BALF) of common variable immunodeficiency (CVID)-interstitial lung disease (ILD) compared to sarcoidosis. Memory CD4 T cells were differentiated into $CXCR5^{pos}$ $T_{FH}1$-, $T_{FH}1/17$-, $T_{FH}17$-, $T_{FH}2$-like cell subsets according to their CXCR3 and CCR6 expression **(A)** and total memory CD4 T cells into $CXCR5^{neg}PD1^{high}$ T_{PH} cells. Shown are two examples with high and low amounts of T_{PH} cells **(B)**. Corresponding statistics are shown below. Memory CD4 T cells were further differentiated into $FoxP3^{+}CD25^{+}$ Tregs, statistics are shown on the right **(C)**. The mean fluorescence intensity (MFI) of FoxP3, CD25, CTLA-4 in Tregs is shown in **(D)** and the ratio of $CXCR5^{pos}$ memory CD4 T_{FH}-like cells to Tregs in **(E)**. Defined genetic defects are marked by color coding. *P <.05, **P <.01 ***P <.001, Sarc., sarcoidosis.

patients when compared to healthy donors (**Figure 4A**) while BAFF, CXCL9, CXCL13, CXCL14, and CXCL10 (IP10) of the same samples were below the detection limit (data not shown).

In an independent subgroup of CVID patients, sarcoidosis patients as well as healthy donors we performed an analysis by MultiPlex Bead Arrays of BAL fluids for CXCL10, IL-4, IL-10, IL-12, and IL-17. IL-10 and IL-12 of most of the samples were below the detection limit and therefore not shown. CXCL10 and IL-17 concentrations were significantly increased in the BALF of CVID patients compared to healthy donors (**Figure 4B**). CXCL10 was also increased in most of the sarcoidosis patients. No differences were observed for IL-4.

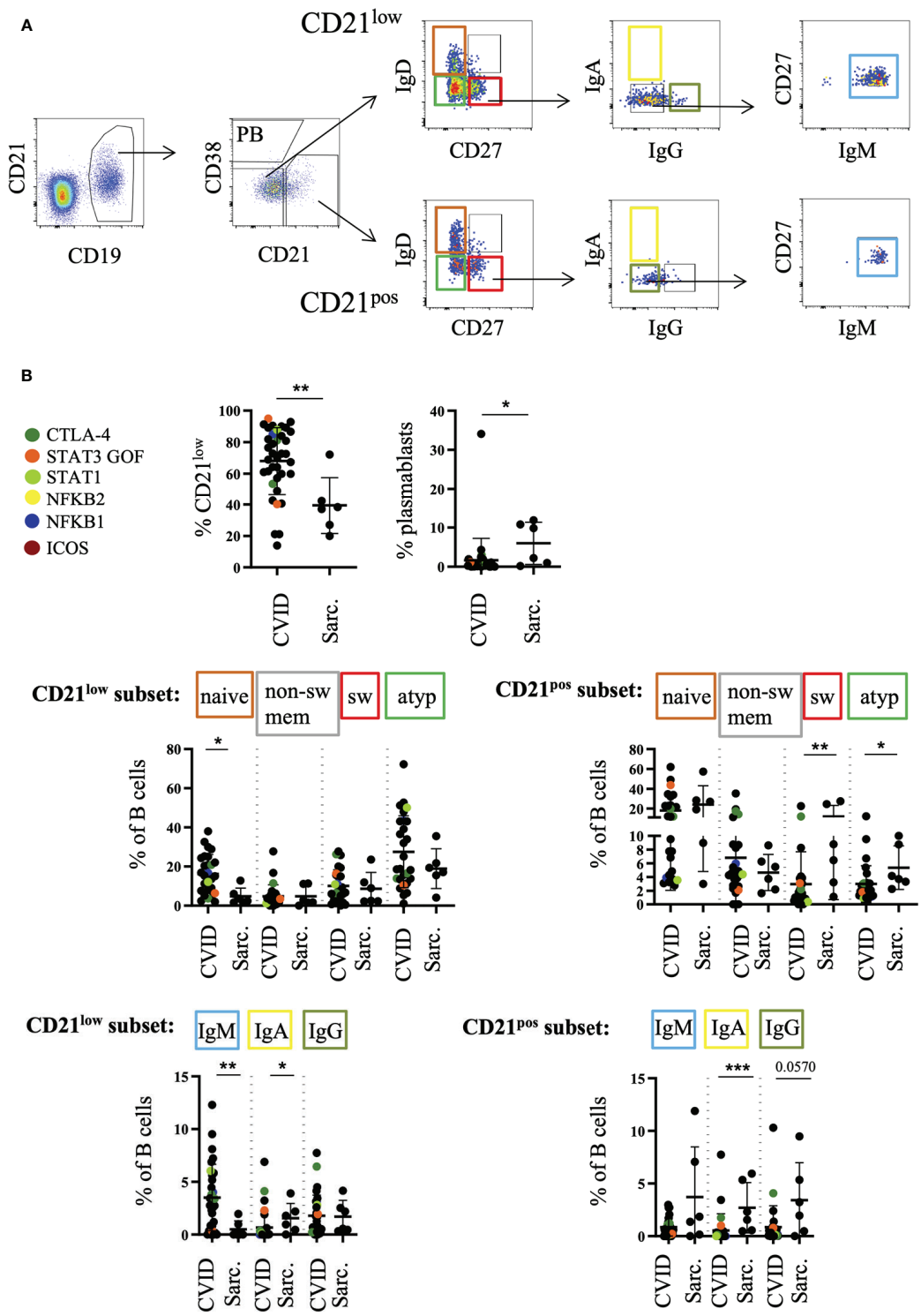

FIGURE 3 | Increased percentage of CD21^{low} cells in bronchoalveolar lavage fluid (BALF) of common variable immunodeficiency (CVID)-interstitial lung disease (ILD) compared to sarcoidosis. B cells were further divided into CD21^{low} B cells, plasmablasts (PB) and CD21^{pos} B cells. An exemplary FACS plot is shown in **(A)**. Naive (IgD^{+}CD27^{-}), switched memory B cells (IgD^{-}CD27^{+}), atypical (IgD^{-}CD27^{-}) and non-switched memory B cells (IgD^{+}CD27^{+}) were gated from the CD21^{low} B-cell compartment as well as from the CD21+ nonPB subset. IgA, IgG, IgM-only cells were gated out of the switched memory B cell gate (IgD^{-}CD27^{+}). Corresponding statistical analysis is shown in **(B)**. Defined genetic defects are marked by color coding. *P <.05, **P <.01 ***P <.001, Sarc, sarcoidosis.

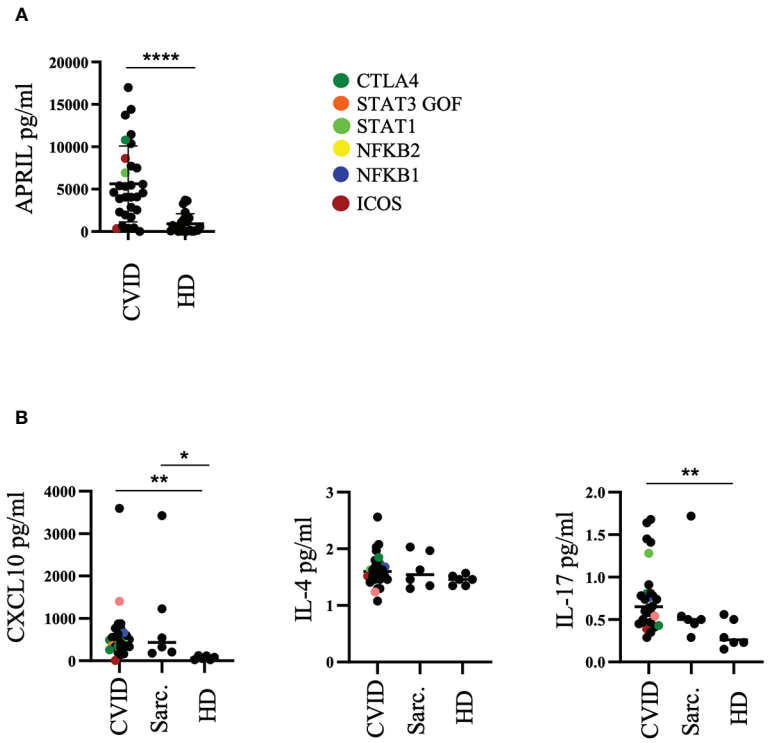

FIGURE 4 | Altered cytokine milieu in bronchoalveolar lavage fluid (BALF) of common variable immunodeficiency (CVID)-interstitial lung disease (ILD). **(A)** ELISA of BALF supernatants for APRIL production. **(B)** Multiplex Bead Array of BALF supernatants for CXCL10, IL-4 and IL-17. Defined genetic defects are marked by color coding. *P <.05, **P <.01 ****P <.0001 HD, healthy control; Sarc, sarcoidosis.

Correlations Between Cell Subsets and Cytokines in BALF and Peripheral Blood of CVID-ILD

In order to integrate the different findings we analyzed the association of the accumulation of different cell types and the concentration of the different cytokines and chemokines in BALF. Increased neutrophil counts in BALF of CVID patients positively correlated with elevated levels of IL-17 (**Figure 5A**). We could neither detect a correlation between CXCL10 and the expansion of $T_{FH}1$, T_{PH} cells or $CD21^{low}$ B cells nor of APRIL with total B cells, switched memory B cells or $CD21^{low}$ B cells (data not shown). There was, however, a strong positive correlation of the percentage of B cells and T_{PH} cells in the BALF (**Figure 5B**), and to a lesser degree between the percentage of $CD21^{low}$ B cells and $T_{FH}1$ cells (**Figure 5C**) which originated from a correlation of IgA^{pos} $CD21^{low}$ B cells and $T_{FH}1$ cells (**Figure 5D**). Interestingly, this was not seen for IgG memory B cells.

When comparing the different T and B cell subsets in peripheral blood and BALF of CVID-ILD patients there were not sufficient data of the extended T cell phenotyping for T_{FH} and T_{PH} in peripheral blood performed at the same time in order to draw firm conclusions. When comparing the B-cell subpopulations however there was a significant correlation of the percentage of total (**Figure 5E**) and naïve $CD21^{low}$ B cells (**Figure 5F**) and of switched memory $CD21^{pos}$ B cells (**Figure 5G**) between both compartments.

DISCUSSION

Interstitial lung disease in patients with CVID is usually characterized by a mixed T- and B-cell infiltrate of the interstitial space (13, 14, 16, 17). Here we could show that this previously reported lymphocytic infiltrate is reflected by the expansion of lymphocytes in the bronchoalveolar space detected in over 80% of the patients. Similar to the histological findings, the majority of the lymphocytes consist of T cells but there is an additional significant expansion of B cells compared to healthy controls and patients with sarcoidosis. Like in peripheral blood, switched memory and especially IgA^{pos} B cells were reduced in BALF of CVID patients compared to sarcoidosis. However, a substantial amount of CVID patients accumulated IgG^{pos} B cells in the BALF despite a profound reduction of IgG^{pos} B cells in blood. As we had previously reported the majority of B cells in the BALF belong to the $CD21^{low}T\text{-}bet^{hi}$ population (29). Also most of the $CD21^{low}$ B cells which can present as naïve, non-switched and switched classical and atypical memory B cells (31), in the BALF of CVID-ILD had a naïve or non-class switched atypical memory

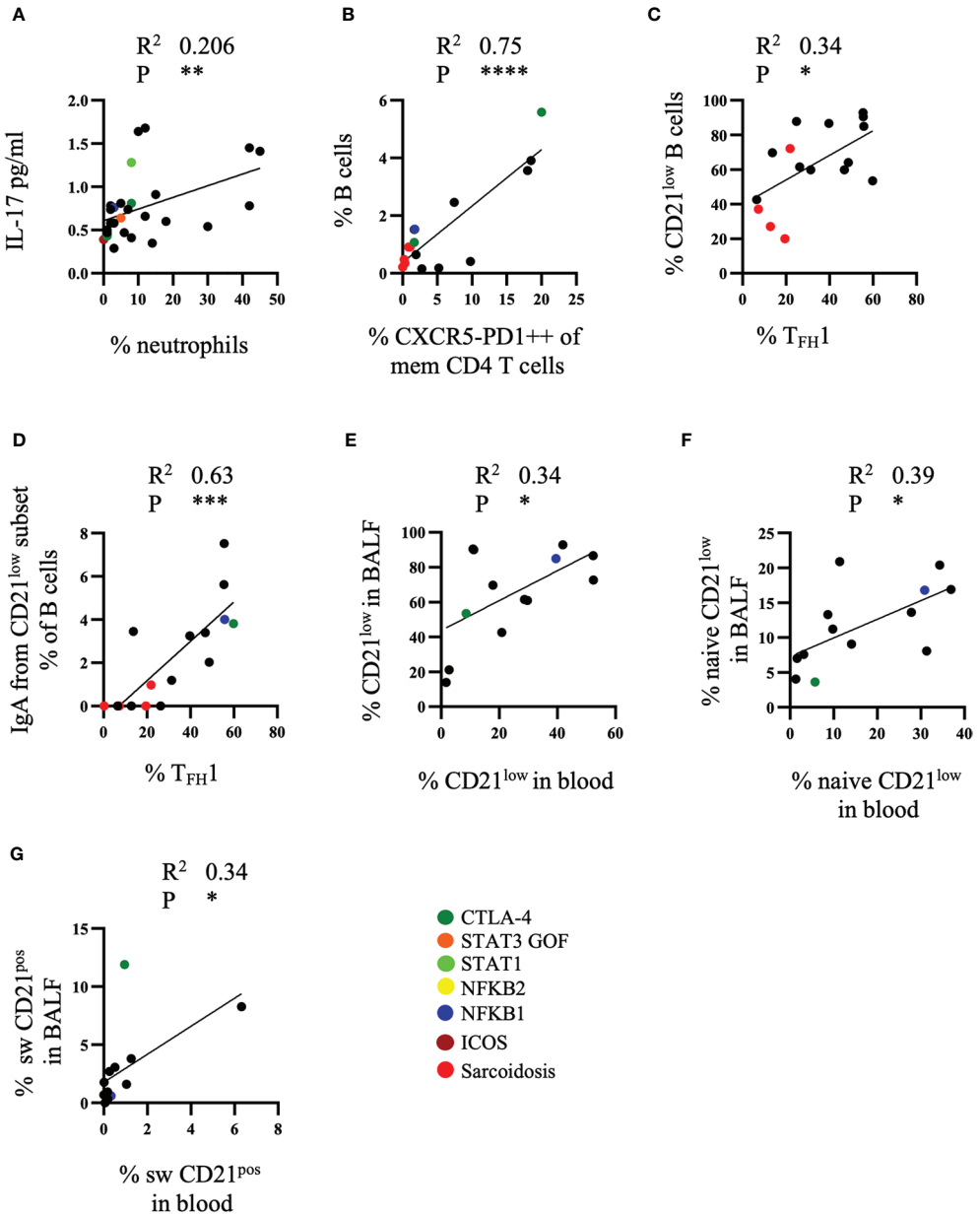

FIGURE 5 | Correlations between cell subsets and cytokines of bronchoalveolar lavage fluid (BALF). **(A)** Correlation of IL-17 in BALF of common variable immunodeficiency (CVID) patients with neutrophil numbers (n = 28), **(B)** of B cells and T_{PH} cells (n = 16), **(C)** of $CD21^{low}$ B cells and $T_{FH}1$ cell subset (n = 16) and **(D)** of IgA^{pos} $CD21^{low}$ B cells and $T_{FH}1$ cells (n = 16). Correlation of total $CD21^{low}$ B cells (n = 13) **(E)**, naïve $CD21^{low}$ B cells (n = 13) **(F)** and switched $CD21^{pos}$ B cells (n = 13) **(G)** in BALF and peripheral blood. Defined genetic defects are marked by color coding. *P <.05, **P <.01 ***P <.001, ****P <.0001.

phenotype. This population is linked to a T_H1 driven inflammatory environment (30) where other costimulatory factors like IL-21 may contribute to their differentiation (32). Compatible with this hypothesis we found an expansion of $T_{FH}1$ cells within the BALF compared to sarcoidosis significantly correlating with the expansion of $CD21^{low}$ B cells, prone to provide both IFNγ and IL-21 co-stimulation. Compatible with the role of T_{FH} cells in memory formation, the percentage of $T_{FH}1$ cells demonstrated a highly significant correlation with the percentage of IgA^{pos} memory B cells among the $CD21^{low}$ B-cell population. T_{FH} cells have not been investigated in the bronchoalveolar space before yet our findings support the presence of tertiary GC in the lung tissue of CVID patients with ILD as reported by Maglione et al. (16, 17) and represent a fundamental difference between sarcoidosis- and CVID-associated ILD given that the BALF of the latter not only contain more B cells but also a higher percentage of T_{FH} cells. Corresponding to the low T_{FH} cell proportion in the BALF in

sarcoidosis, to our knowledge no tertiary GC formation in the lung has been described in this disease condition. Interestingly, unlike CVID-ILD B cell infiltrates of the inter-granulomatous lung tissue are not reflected in the BALF of sarcoidosis patients (33).

In addition to the relative expansion of $T_{FH}1$ cells reflecting GC activity, there was a significant expansion of the recently discovered T_{PH} cells in BALF of CVID patients with ILD. These cells have a similar capacity like T_{FH} cells in co-stimulation of B cells but are usually found in peripheral tissues without bona fide GC activity. They have been described in the synovium of patients with active rheumatoid arthritis (28), in inflamed intestinal tissues in Crohn's disease (34), IgG4-related diseases (35, 36), systemic sclerosis (37), IgA nephropathy (38), type I diabetes (39) and most likely within loose lymphocytic aggregates of murine airway inflammation models (40) but also expanded in peripheral blood of rheumatoid arthritis, systemic lupus erythematosus (SLE) and in Sjögren's syndrome (41–45). Potentially, T_{PH} cells may drive the differentiation of B cells in the less organized inflammatory tissue structures of the lymphocytic infiltrates of the lung (46). Given their capacity for IL-21 and IFNγ production T_{PH} cells are good candidates inducing the differentiation of $CD21^{low}$T-bet^{hi} B cells in peripheral tissues. Especially the "atypical memory" $CD21^{low}$ B-cell population as the largest population in BALF of many CVID-ILD patients might be the main target B-cell population of T_{PH} cell interaction in the lung as had been previously suggested in lupus (47–49). We found a highly significant correlation of T_{PH} cells and B cells in the BALF of patients. Similarly, both $CD11c^{+}CD21^{-}CXCR5^{-}$ B cells and T_{PH} cells were found increased in lupus nephritis tissues (50, 51). Furthermore the frequency of both cell subsets is highly associated in blood of SLE patients (43, 50).

The analysis of cytokines confirmed an environment supporting T_H1-driven inflammation and B cell survival and expansion. While we could detect only very low levels of BAFF which had previously been described as an important cytokine in the BALF of CVID-ILD patients (17) we detected high levels of APRIL. This factor may not only allow for local B-cell survival but may actually contribute to the differentiation of the detectable class switched memory B cells as it has the capacity to support class switch in mucosal tissues (52). It is tempting to speculate whether relevant ILD is less common in TACI deficient patients (53) despite the presence of lymphoproliferation and autoimmunity, two manifestations predisposing for ILD in CVID. The increased levels of IL-12 in some patients demonstrate a potential bias of non-lymphocytic cells like local macrophages endorsing the T_H1 environment. Similar to sarcoidosis CXCL10 is significantly elevated in CVID-ILD derived BALF being one of the main chemokines attracting not only CXCR3 positive T_H1 cells but also $CD21^{low}$T-bet^{hi} B cells which likewise express high levels of this chemokine receptor (30). Interestingly, unlike the gastrointestinal tissue (54) we could also detect elevated IL-17 concentration in some of the CVID-ILD BALF. Given the reduction of T_H17 cells in the BALF of CVID patients IL-17 must be mainly produced by $T_H1/17$ cells. Increased IL-17 concentrations were associated with an increased proportion of neutrophils in the BALF as IL-17 supports their recruitment. This seems to be frequently driven by additional bacterial airway infection.

When comparing the lymphocyte subsets circulating in peripheral blood with the subsets in BALF, we did not have sufficient data on T cell populations in order to draw definite conclusions, but among B cells there was a significant correlation between the percentage of total and naïve $CD21^{low}$ B cells and switched memory $CD21^{pos}$ B cells in both compartments. While the first most likely reflects a direct communication between both pools, we assume that the correlation of the percentage of switched memory B cells rather reflects the general capacity of the patient to class switch. In order to confirm these assumptions, BCR sequencing of both compartments is required in order to determine clonal relationship.

Future studies will also need to perform direct comparison of BALF and histology of lung tissue in order to determine how much the changes we could demonstrate in BALF in this study truly reflect the pathology in the tissue. Such studies will require in depth phenotyping of T and B cells, including TCR and BCR sequencing to demonstrate clonal relationship between the lymphocyte populations, a careful evaluation of the cytokine milieu and foremost the sensitivity of BALF analysis for lymphoma as a differential diagnosis in ILD of CVID.

In summary, BALF of CVID patients with ILD is mainly characterized by an expansion of lymphocytes. Unlike in sarcoidosis these consist of a mixed T- and B-cell expansion reflecting the mixed infiltrates in lung tissue of CVID-ILD patients. The simultaneous expansion of $CD21^{low}$T-bet^{hi} B cells, $T_{FH}1$ and T_{PH} cells in the BALF of CVID-ILD strongly points toward cognate interactions of these populations potentially in tertiary GCs driving the lymphocytic interstitial pneumonitis often seen in these patients. This hypothesis is supported by the cytokine milieu identified in the BALF. Based on these findings it will be of high interest to test whether detailed analysis of BALF sufficiently reflects the pathology of the lung tissue in order to potentially render BALF analysis a valuable tool in diagnosing the presence and activity of ILD in CVID and guide treatment decisions.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of the University Medical Center Freiburg, Freiburg, Germany. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

SU, MR, BK, and DF performed experiments and analyzed the data. DF wrote the first draft of the manuscript. SG provided clinical data. JS and AP supervised ELISAs of BALF. GZ, BF, and AP provided BAL samples. KW devised and supervised the study, designed the research, and edited the manuscript. All authors corrected the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

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Granulomatous Lymphocytic Interstitial Lung Disease (GLILD) in Common Variable Immunodeficiency (CVID): A Multicenter Retrospective Study of Patients From Italian PID Referral Centers

Francesco Cinetto[1,2†], Riccardo Scarpa[1,2†], Maria Carrabba[3], Davide Firinu[4], Vassilios Lougaris[5,6], Helena Buso[1,2], Giulia Garzi[7], Sabrina Gianese[1,2], Valentina Soccodato[7], Alessandra Punziano[8], Gianluca Lagnese[8], Giulio Tessarin[5,6], Giulia Costanzo[4], Nicholas Landini[9], Stefania Vio[10], Maria Pia Bondioni[11], Dario Consonni[12], Carolina Marasco[13], Stefano Del Giacco[4], Marcello Rattazzi[1,2], Angelo Vacca[13], Alessandro Plebani[5,6], Giovanna Fabio[3], Giuseppe Spadaro[8], Carlo Agostini[1,2], Isabella Quinti[14] and Cinzia Milito[14]*

[1] Department of Medicine—DIMED, University of Padova, Padua, Italy, [2] Internal Medicine I, Ca' Foncello Hospital, AULSS2 Marca Trevigiana, Treviso, Italy, [3] Internal Medicine Department, Rare Disease Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy, [4] Department of Medical Sciences and Public Health, University of Cagliari, Monserrato, Italy, [5] Department of Clinical and Experimental Sciences, Pediatrics Clinic and Institute for Molecular Medicine A. Nocivelli, University of Brescia, Brescia, Italy, [6] ASST-Spedali Civili di Brescia, Brescia, Italy, [7] Regional Reference Centre for Primary Immune Deficiencies, Azienda Ospedaliera-Universitaria Policlinico Umberto I, Rome, Italy, [8] Department of Translational Medical Sciences—Center for Basic and Clinical Immunology Research, University of Naples Federico II, Naples, Italy, [9] Radiology Unit, Ca' Foncello Hospital, AULSS2 Marca Trevigiana, Treviso, Italy, [10] Radiology Unit, Azienda Ospedaliera di Padova, Padova, Italy, [11] Radiology Unit, ASST-Spedali Civili di Brescia, Brescia, Italy, [12] Epidemiology Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy, [13] Department of Biomedical Sciences and Human Oncology, Section of Internal Medicine and Clinical Oncology, University of Bari Medical School, Bari, Italy, [14] Department of Molecular Medicine, "Sapienza" University of Rome, Rome, Italy

Background: Granulomatous and Lymphocytic Interstitial Lung Diseases (GLILD) is a severe non-infectious complication of Common Variable Immunodeficiency (CVID), often associated with extrapulmonary involvement. Due to a poorly understood pathogenesis, GLILD diagnosis and management criteria still lack consensus. Accordingly, it is a relevant cause of long-term loss of respiratory function and is closely associated with a markedly reduced survival. The aim of this study was to describe clinical, immunological, laboratory and functional features of GLILD, whose combination in a predictive model might allow a timely diagnosis.

Methods: In a multicenter retrospective cross-sectional study we enrolled 73 CVID patients with radiologic features of interstitial lung disease (ILD) associated to CVID (CVID-ILD) and 125 CVID patients without ILD (controls). Of the 73 CVID-ILD patients, 47 received a definite GLILD diagnosis while 26 received a clinical-radiologic diagnosis of CVID related ILD defined as uILD.

Results: In GLILD group we found a higher prevalence of splenomegaly (84.8 vs. 39.2%), autoimmune cytopenia (59.6 vs. 6.4%) and bronchiectasis (72.3 vs. 28%),

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James Wesley Verbsky, Medical College of Wisconsin, United States

***Correspondence:**
Francesco Cinetto
francesco.cine@gmail.com

[†] These authors have contributed equally to this work

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and lower IgA and IgG serum levels at CVID diagnosis. GLILD patients presented lower percentage of switched-memory B cells and marginal zone B cells, and a marked increase in the percentage of circulating CD21lo B cells (14.2 vs. 2.9%). GLILD patients also showed lower total lung capacity (TLC 87.5 vs. 5.0%) and gas transfer (DLCO 61.5 vs. 5.0%) percent of predicted. By univariate logistic regression analysis, we found IgG and IgA levels at CVID diagnosis, presence of splenomegaly and autoimmune cytopenia, CD21lo B cells percentage, TLC and DCLO percent of predicted to be associated to GLILD. The joint analysis of four variables (CD21lo B cells percentage, autoimmune cytopenia, splenomegaly and DLCO percent of predicted), together in a multiple logistic regression model, yielded an area under the ROC curve (AUC) of 0.98 (95% CI: 0.95-1.0). The AUC was only slightly modified when pooling together GLILD and ulLD patients (0.92, 95% CI: 0.87-0.97).

Conclusions: we propose the combination of two clinical parameters (splenomegaly and autoimmune cytopenia), one lung function index (DLCO%) and one immunologic variable (CD21lo%) as a promising tool for early identification of CVID patients with interstitial lung disease, limiting the use of aggressive diagnostic procedures.

Keywords: GLILD, CVID-ILD, CD21lo B cells, splenomegaly, autoimmune cytopenia, DLCO

INTRODUCTION

Common Variable Immunodeficiency (CVIDs) is the most commonly diagnosed (1), clinically relevant primary antibody deficiency characterized by both infectious and non-infectious complications. The introduction of intravenous or subcutaneous, immunoglobulin replacement therapy has markedly decreased morbidity and mortality due to infection (2, 3). In contrast, non-infectious complications, such as autoimmune manifestations, cytopenias, inflammation, lung disease, lymphoproliferation, and malignancies result increased, involving almost 70% of patients (4). The presence of non-infectious complications is associated with more severe prognosis and reduced quality of life (5–7).

Up to 90% of CVID patients may develop lung complications such as infection-related, immune-mediated and neoplastic diseases (8). Among these, Granulomatous and Lymphocytic Interstitial Lung Diseases (GLILD) is a severe non-infectious complication, reported in around 8–20% of cases (9, 10). GLILD has been defined as "a distinct clinico-radio-pathological ILD occurring in patients with CVID, associated with a lymphocytic infiltrate and/or granuloma in the lung, and in whom other conditions have been considered and where possible excluded" (9). It is a relevant cause of long-term lung damage and impairment of respiratory function and it is closely associated with poor clinical outcomes (5, 8, 11, 12) At present, although the pathogenesis of GLILD is still far from being understood, it may be considered as a manifestation of immune dysregulation (13), as also underlined by the increased frequency of other immune-mediated CVID complications in GLILD patients (14).

Based on UK-PID Network Consensus, current diagnostic recommendations in the suspicion of GLILD include chest CT scan, lung function tests (PFTs), bronchoscopy and a surgical lung biopsy, this latter mandatory to put a definite diagnosis (9). Several epidemiologic studies have underlined that the risk of performing a lung biopsy is clinically relevant and this risk increases with age, disease severity, or comorbidities (15). Moreover, GLILD in some cases may be misdiagnosed as granulomatous lung disease of other nature.

The possibility to define clinical, laboratory and radiological parameters that may identify CVID patients at high risk for GLILD development or allow for early diagnosis, might limit the use of lung biopsy and related risks and will potentially ameliorate affected patients' prognosis.

In addition, the introduction of MRI may represent a reliable radiation-free technique for diagnosis and follow-up of GLILD patients (16, 17), associated with evaluation of the broncho-alveolar lavage (BAL) in terms of GLILD related markers such as inflammatory cytokines and lymphocyte subsets (18).

Over the last years, different data have been reported on GLILD patients, suggesting that they are characterized by reduced overall survival and tend to develop an immune dysregulation including splenomegaly, lymphoproliferation and autoimmune cytopenias (12, 19, 20). Kellner et al. reported that patients with chronic lung disease had lower T cell counts and increased prevalence of non-bacterial infections in addition to autoimmune cytopenia (7) whereas Mannina et al. defined hypersplenism and polyarthritis as strong risk factors for GLILD (21). Finally, Hartono et al. proposed a GLILD predictive model based on splenomegaly, CD21lo B cells percentage, autoimmune cytopenia and serum IgA levels (14).

Nonetheless, to date, there is a lack of well-defined clinical, laboratory, and radiological parameters that may identify a clinical phenotype of patients affected by GLILD or prone to its development. With the aim to overcome this gap, we undertook this multicenter observational retrospective study in order to describe clinical, immunological, laboratory, and radiological

features of GLILD patients that may lead to the identification of specific features and possible biological predictors capable of allowing an early diagnosis in patients at high risk to develop ILD (14).

MATERIALS AND METHODS

Study Population

We conducted a multicenter retrospective cross-sectional study in which we enrolled patients with a diagnosis of CVID with interstitial lung disease (defined as CVID-ILD) and without it (defined as controls) from 7 Italian adult Italian Primary Immune Deficiency Network (IPINET) referral centers (Rome, Treviso, Milan, Brescia, Naples, Cagliari, Bari). Each center provided at least one age-matched control for each CVID-ILD patient.

All participants were enrolled in the IPINET Registry. This study was approved by the local institutional review board and was performed in accordance with the Declaration of Helsinki. All participants signed the written informed consent form prior to inclusion in the study.

Inclusion criteria were:

1) CVID diagnosis according to the ESID registry working party (22) with at least 18 months of follow-up since diagnosis;

2) For the subgroup of CVID-ILD patients a chest HRCT scan consistent with ILD according to existing literature, a bronchoalveolar lavage excluding and infectious interstitial pneumonia and:

- Either CVID-ILD diagnosis based on video-assisted thoracoscopic surgery (VATS) or transbronchial biopsy, or on lymph nodal or other organ's biopsy excluding B-cell malignancy. This group was defined as **GLILD** (9, 19, 23)
- **Or** CVID-ILD diagnosis obtained by clinical, functional and radiologic evaluation, in which no suspicion of B cell malignancy could be raised, a lung biopsy for histological diagnosis was too dangerous, or refused by the patients, or resulted no conclusive for GLILD. Patients belonging to this group were defined as undefined Interstitial Lung Disease **(uILD)**.

All CVID-ILD patients received a final diagnosis of **GLILD** or **uILD** after a multidisciplinary team discussion involving experienced Clinical Immunologists, lung Radiologists, Pathologists, Pulmonologists, with participation of Hematologists and Infectious disease Specialists when required (24).

For CVID-ILD and controls, the following reports had to be available at enrollment: at least one HRCT scan, 2 abdominal ultrasounds, IgG, IgA, and IgM levels at diagnosis and at last follow up, clinical history regarding cancer, enteropathy, autoimmune cytopenia, lymphoproliferation, smoking status, CD19+, and B lymphocytes subsets.

Data Collection

The retrospective examination of clinical records of all enrolled subjects (GLILD patients, uILD patients and controls) aimed to investigate:

- Demographic parameters (Age, sex, BMI, smoking status, age at CVID diagnosis, diagnostic delay);
- Clinical phenotypes according to the revised Chapel et al. classification (5);
- Presence or absence of splenomegaly (defined as a spleen enlargement confirmed by two abdominal ultrasound and/or CT scan and/or MRI repeated at least 12 months apart from each other according to the Radiologist performing the test), bronchiectasis, autoimmunity, cancer;
- Laboratory parameters: IgG, IgA, IgM at CVID diagnosis and at last follow-up visit; for IgG, trough level (IgGTL) has been considered under replacement therapy
- Lymphocyte subsets according to Euroclass classification (25)
- Route and dosage of immunoglobulin replacement therapy (IgRT)
- Lung function, including 1st second Forced Expiratory Volume (FEV1), Forced Vital Capacity (FVC), Total Lung Capacity (TLC), and gas transfer (DLCO). Data were expressed as percent of predicted, according to ATS guidelines.
- Lung HRCT scan picture

In addition, for GLILD patients:

- Histology, site of biopsy
- 6-min walking test (distance, symptoms/desaturation), when available
- Broncho-alveolar lavage fluid (BALF) flow cytometry results, when available.

HRCT Analysis

Blind HRCT scan evaluation was performed by three lung radiologists in a subgroup of GLILD patients and controls, in order to compare airways and parenchymal abnormalities. The following parameters were registered, scored in terms of absence/presence: bronchiectasis, bronchial wall thickening, mucus plugging, and centrilobular nodules, solid nodular opacities, excavated opacities, ground glass opacities <5 mm and >5 mm, consolidations, Halo sign, linear opacities, signs of fibrosis, mosaic attenuation, emphysema, lymph nodes increase in number and/or size, lymph nodes calcifications. Moreover, with the limits due to the possible non-complete inclusion of the whole spleen and liver (in particular) parenchyma in the scan, evidence of splenomegaly at caudal sections of HRCT scan was registered. Differences were resolved by consensus. For GLILD patients, HRCT scan images used for comparison were all acquired at GLILD diagnosis or at least before GLILD treatment. The list of radiological findings was defined on the basis of existing literature and clinical experience (26, 27). The syllabus of the Fleischner Society was used as a cornerstone for the radiological terminology, since the correspondence between images and definitions is well defined and widely accepted (28).

Statistical Analysis

We used Wilcoxon rank-sum (Mann-Whitney) or Kruskal-Wallis test to compare quantitative variables across two or more groups, respectively. We reported median and interquartile range (IQR) as descriptive statistics. Chi-squared and Fisher's exact tests were used for categorical variables. Univariate and

multivariable logistic regression models were fitted to calculate odds ratios (OR), 95% confidence intervals (CI) and area under the curve (AUC) of receiver operating characteristic (ROC) curves. Variables entered in the multivariable model were chosen based either on clinical grounds and existing literature or on results of univariate models. Statistical analyses were performed with Stata 16 (StataCorp.2019).

RESULTS

We enrolled 73 CVID patients with radiologic features of CVID-ILD and 125 CVID patients without ILD (controls). Of the 73 ILD patients 47 received a definite GLILD diagnosis while 26 were classified as uILD.

All patients were regularly treated with adequate substitutive treatment using polyvalent IgGs. 77.6% of controls, 87.23% of GLILD and 88.46% of uILD were under subcutaneous replacement therapy (SCIg). A total of 104 out of 198 patients performed a genetic screening: 2 patients with ILD presented a TACI mutation, as well as 3 controls, and 5 patients with CVID-ILD presented a CTLA4 mutation (3 with histologic diagnosis of GLILD, 1 with a clinical-radiologic diagnosis of uILD). Other genetic variants were detected in 1 control, 3 GLILD and 2 ILD patients. The screening for CVID associated genes is currently ongoing.

Demographic parameters are summarized in **Table 1**. No statistically significant differences were detected for controls and CVID-ILD patients in terms of sex, age, age at CVID diagnosis, age at CVID onset, and diagnostic delay. When focusing on CVID-ILD patients, uILD patients showed older age at CVID onset and a more recent CVID diagnosis when compared to GLILD patients. Median age at enrollment was 46, 47, and 49.5 years for controls, GLILD and uILD, respectively. There was a prevalence of female sex between controls and CVID-ILD patients (56 vs. 70%, respectively); the percentage of female patients was lower in uILD than in GLILD, but without statistical significance. Moreover, there was no difference between groups in terms of body weight, BMI and smoking status. A further description of the CVID-ILD population is available in the **Supplementary Material**. We will first present the results of the comparison between the control group and the GLILD group; finally, we will discuss similarities and differences between the GLILD and uILD subgroups, focusing on the role of clinical predictors in the diagnostic process.

Clinical Phenotype

We then compared GLILD and controls in terms of clinical phenotypes according to Chapel et al. (5) (**Table 2**). Control group included a significantly higher percentage of patients presenting the "infection only" phenotype (70.4 vs. 2.12%, $p < 0.0001$), while the GLILD group was characterized by an increased frequency of the lymphoproliferation and cytopenia phenotypes ($p < 0.0001$). No difference was detected in terms of enteropathy and cancer, being cancer borderline higher in the GLILD group. When considering the different types of cancer, the only significant difference was registered in the prevalence of T and B clonal lymphoproliferative diseases (B cell Non-Hodgkin lymphomas and T-large granular lymphocyte leukemia T-LGLL). Interestingly, a clear difference was detected between GLILD and controls when comparing the prevalence of bronchiectasis ($p < 0.0001$), splenomegaly ($p < 0.0001$) and idiopathic thrombocytopenic purpura ($p < 0.0001$). Of note, 8 of 47 GLILD patients and 3 of 26 uILD had previously undergone splenectomy, due to autoimmune cytopenia. Moreover, 5 Evans' syndromes were identified in GLILD, 1 in controls, none in uILD. In line with the higher prevalence of bronchiectasis, GLILD patients more frequently underwent antibiotic prophylaxis ($p < 0.0001$), that was almost performed with azithromycin 250 mg/die for 3 consecutive days per week, while in only 2 patients (belonging to the control group) with trimethoprim/sulfametoxazole (**Table 1**).

Ig Serum Levels, IgG Trough Level, and Ig Replacement Therapy

IgG serum levels at the time of CVID diagnosis were found significantly lower both in GLILD (IgG 241.0 mg/dl, IQR 79.0-382.0) and in uILD (230.0 mg/dl, IQR 109-307) than in controls (349 mg/dl, IQR 167.0-451.0) ($p < 0.05$). The same was observed for IgA (**GLILD** 8.0 mg/dl, IQR 1.2-21.0; **uILD** 6.0 mg/dl, IQR 5.0-9.5; **controls** 17.0 mg dl, IQR 6.0-29.5; $p < 0.01$). No difference was found in IgM levels at diagnosis and at last follow-up. (**GLILD** 19.0 mg/dl, IQR 4.0-35.0; **uILD** 9.5 mg/dl, IQR 5.0-30.0; **controls** 21.5 mg/dl, IQR 10.0-41-0 at diagnosis) ($p > 0.05$); (**GLILD** 20.0 mg/dl, IQR 4.0-50.0; **uILD** 15.5 mg/dl, IQR 4.5-41; controls 22.0 mg/dl, IQR 5.0-46.0 at last follow-up ($p > 0.05$). Only 2 patients presented an increase in polyclonal IgM levels after CVID-ILD diagnosis, one with GLILD and one with uILD. The difference in IgA serum level was confirmed at last FU; IgG trough levels were similar in GLILD, uILD and controls (**GLILD** 799.2 mg/dl, IQR 677.5-933.5; **uILD** 833.0 mg/dl, IQR 733.0-944.5; **controls** 796.5 mg/dl, IQR 669.0-937.5) ($p > 0.05$) (**Supplementary Figure 1**).

GLILD and uILD patients required higher dosage of IgRT than controls to achieve similar IgG trough levels (**GLILD** 400.0 mg/kg -IQR 350-480; **uILD** 402.0 mg/kg, IQR 380-500; **controls** 365.4 mg/kg -IQR 274.3-444.0) ($p < 0.05$) (**Supplementary Figure 1**). No differences were found between GLILD and uILD for any of the Ig-related measures. There was no difference in route of Ig administration between groups.

Lymphocytes Subsets

CVID patients with and without GLILD were then compared analyzing B and T cell subsets before immunosuppressive treatment. There were no differences in lymphocytes absolute count and percentage. CD19+ B cell absolute value and percentage was similar in the two groups; the prevalence of patients with <1% of circulating B cells was also superimposable (16.67% GLILD, 12.0% controls). 60.6% of GLILD patients and 44.09% of controls presented <2% of switched-memory B cells (SmB), with no significant difference; however, when comparing SmB percentage of B cells, GLILD patients presented lower values than controls ($p < 0.05$). GLILD patients also showed a lower percentage of marginal zone B cells (MZB) than controls ($p < 0.05$). No differences were found in distribution of plasmablasts,

TABLE 1 | Characteristics of the population.

	Controls $n = 125$ median (IQR)	GLILD $n = 47$ median (IQR)	uILD $n = 26$ median (IQR)	*p value* (GLILD vs. ctrls)	*p value* (uILD vs. GLILD)	*p value* (uILD vs. ctrls)
Sex F (*n*; %)	70 (56.0%)	33 (70.2%)	13 (50.0%)	0.11	0.12	0.66
Age (years)	46 (34–59)	47 (37–60)	49.5 (43–61)	0.96	0.31	0.44
Age at CVID onset	28 (13.0–38.0)	21 (13.0–36.0)	38.5 (18.0–48.0)	0.36	**0.02**	0.07
Age at CVID diagnosis	37 (26.0–46.0)	35 (27.0–46.0)	42 (33.0–52.0)	0.92	0.16	0.25
Diagnostic delay (years)	6 (2.0–13.0)	6 (2.0–16.0)	5.5 (3.0–10–0)	0.52	0.45	0.31
Years since CVID onset	18 (10.0–27.0)	18 (11.0–33.0)	14 (8.0–20.0)	0.40	**0.02**	0.12
Body weight (Kg)	67.0 (56.8–82.0)	62.0 (59.0–75.0)	61.5 (56.0–77.0)	0.26	0.76	0.11
BMI	24.6 (21.3–27.8)	23.7 (21.4–26.0)	22.7 (20.5–27.2)	0.26	0.97	0.14
Current or former smoker (*n*; %)	29 (24.4%)	14 (29.8%)	4 (15.4%)	0.55	0.25	0.44
Antibiotic prophylaxis (*n*; %)	32 (25.6%)	22 (46.8%)	7 (26.9%)	**0.0099**	0.09	0.63

TABLE 2 | Chapel's phenotypes I-IV and other disease-related complications.

	Controls $n = 125$ *n* (%)	GLILD $n = 47$ *n* (%)	uILD $n = 26$ *n* (%)	*p value* (GLILD vs. ctrls)	*p value* (uILD vs. GLILD)	*p value* (uILD vs. ctrls)
Infections only (I)	88 (70.4)	1 (2.1)	8 (30.8)	**<0.0001**	**<0.001**	**<0.001**
Cytopenia (II)	13 (10.4)	29 (61.7)	8 (30.8)	**<0.0001**	**0.015**	**0.012**
Lymphoproliferation (III)	28 (22.4)	43 (91.5)	16 (61.5)	**<0.0001**	**0.004**	**<0.0001**
Enteropathy (IV)	14 (11.2)	9 (19.1)	6 (23.1)	0.20	0.76	0.11
Cancer	18 (14.4)	12 (25.5)	4 (15.4)	0.086	0.38	1.0
B-cell lymphoma	5 (4.0)	5 (10.6)	2 (7.7)	0.097	0.68	0.41
B-cell Lymphoma & T-LGLL	5 (4.0)	9 (19.1)	3 (11.5)	**0.001**	0.40	0.14
Splenomegaly	49 (39.2)	39 (84.8)	20 (70.0)	**<0.0001**	0.52	**<0.0001**
Bronchiectasis	35 (28.0)	34 (72.3)	15 (57.7)	**<0.0001**	0.20	**0.003**
ITP	8 (6.4)	26 (55.3)	8 (30.8)	**<0.0001**	**0.044**	**0.0018**
AI cytopenia (AIHA+ITP)	13 (10.4)	28 (59.6)	8 (30.8)	**<0.0001**	**0.027**	**0.012**
Autoimmunity	35 (28.0)	32 (68.1)	11 (42.3)	**<0.0001**	**0.047**	0.16

AI cytopenia, history of ITP and/or AIHA.

naïve and transitional B cells. Of note, GLILD patients showed a significant increase in the percentage of circulating CD21lo B cells compared to controls ($p < 0.0001$) (**Table 3**).

When analyzing T cells, GLILD patients presented a lower percentage of CD8+ T cells if compared to controls ($p < 0.01$), with an increased CD4/CD8 ratio ($p < 0.05$). Of note, GLILD patients presented a borderline significant expansion of CD3CD8CD57+ large T granular lymphocytes ($p = 0.06$), becoming significant when pooling together uILD and GLILD subgroups ($p < 0.01$) (**Supplementary Table 1**).

Lung Function

As lung function parameters, according to data availability in clinical records, we considered 1st second Forced Expiratory Volume (FEV1), Forced Vital Capacity (FVC), Total Lung Capacity (TLC), and gas transfer (DLCO). Data were collected before starting any GLILD-specific treatment, as absolute values and percent of predicted. GLILD patients showed a significantly lower FEV1, FVC, TLC, and DLCO compared to controls. When adjusted for disease duration, differences in FEV1 ($p = 0.006$), FVC ($p < 0.001$), TLC% (p 0.001), and DLCO% ($p < 0.001$) were still significant between GLILD and controls (**Table 4**).

HRCT

Since the presence of specific CVID-ILD features represented an Inclusion Criteria both for GLILD and uILD group, there were no differences between these two groups at HRCT scan. HRCT scan evaluation by three experienced lung radiologists was then performed in a subgroup of 26/47 GLILD patients and 26/125 controls, in order to confirm the appropriate selection. Airways and parenchymal abnormalities were evaluated (**Supplementary Table 2**). As expected, a statistically significant difference in favor of GLILD patients was detected in terms of Bronchiectasis ($p < 0.05$), solid nodular opacities ($p < 0.01$), ground glass opacities <5 mm ($p < 0.01$) and >5 mm ($p < 0.001$), consolidations ($p < 0.0001$), halo sign ($p < 0.0001$), linear opacities ($p < 0.0001$), signs of fibrosis ($p < 0.0001$), mosaic attenuation ($p < 0.05$), lymph nodes increase in number ($p < 0.001$) and size (>1 cm) ($p < 0.0001$, absent in control group). Lymph nodes calcifications and excavated opacities were present in only one and two GLILD patients,

TABLE 3 | B Lymphocytes subsets.

	Controls $n = 125$ Median (IQR)	GLILD $n = 47$ Median (IQR)	uILD $n = 26$ Median (IQR)	*p value* (GLILD vs. ctrls)	*p value* (uILD vs. GLILD)	*p value* (uILD vs. ctrls)
Lymphocytes %	29.0 (22.3–37.6)	27.8 (21.8–39.4)	29.0 (26.0–35.0)	0.87	0.54	0.93
Lymphocytes count	2.05 (1.4–2.6)	1.54 (0.99–2.57)	1.87 (1.10–2.30)	**0.02**	0.67	0.44
CD19+ B cells (% of lymphocytes)	7.0 (3.0–12.6)	6.0 (3.0–10.0)	4.0 (2.0–11–0)	0.91	0.17	0.32
Naïve (% of B cells)	72 (56.6–86.0)	81.2 (54.2–88.2)	75.9 (52.0–81.2)	0.48	0.10	0.63
Switched memory (% of B cells)	2.5 (1.0–6.6)	1.3 (0.1–5.0)	3.0 (0.6–5.9)	**0.043**	0.20	0.71
Marginal zone (% of B cells)	11.1 (2.4–24.6)	3.5 (1.3–11.0)	8.0 (5.0–16.7)	**0.043**	0.27	0.48
Transitional (% of B cells)	1.0 (0.2–2.5)	0.6 (0.0–4.0)	2.7 (0.6–7.5)	0.94	0.14	0.09
Plasmablasts (% of B cells)	0.1 (0.0–0.8)	0.3 (0.0–1.1)	0.1 (0.0–1.2)	0.11	0.47	0.66
CD21lo (% of B cells)	3.9 (1.9–7.7)	14.2 (10.1–30.0)	6.0 (2.8–29.3)	**<0.0001**	0.24	0.051

B cells sub-populations are identified according to EUROclass study: Naïve $IgD^+IgM^+CD27^-$; Switched memory $IgD^-IgM^-CD27^+$; Marginal zone $IgD^+IgM^+CD27^+$; Transitional $CD38^{++}IgM^{high}$; Activated $CD21^{low}CD38^{low}$; Plasmablasts $CD38^{+++}IgM^-$(25).

TABLE 4 | Lung function parameters.

	Controls $n = 125$ Median (IQR)	GLILD $n = 47$ Median (IQR)	uILD $n = 26$ Median (IQR)	*p value* (GLILD vs. ctrls)	*p value* (uILD vs. GLILD)	*p value* (uILD vs. ctrls)
FEV1 (% of predicted)	102 (89–111)	88 (72–105)	103 (89–110)	**0.02**	**0.03**	0.94
				0.006	0.15	0.35
FVC (% of predicted)	104 (92–116)	88 (72–103)	104 (93–113)	**<0.001**	**0.01**	0.72
				<0.001	**0.01**	0.40
TLC (% of predicted)	102 (94–108)	87 (75–102)	93 (87–104)	**<0.001**	0.32	**0.03**
				0.001	0.29	**0.05**
DLCO (% of predicted)	83 (75–97)	61 (52–80)	73 (65–86)	**<0.001**	**0.008**	**0.02**
				<0.001	**0.02**	0.07

For each cell: Upper p-value from Wilcoxon-Mann-Whitney test. Lower p value from linear regression models adjusted for disease duration (difference between age at enrolment and age at CVID onset).

respectively, and absent in controls. Moreover, detection of splenomegaly at caudal sections of HRCT scan was significantly higher in GLILD patients ($p < 0.05$), being 2/26 GLILD patients already splenectomized at the time of imaging acquisition; no difference was found in the prevalence of hepatomegaly in the same sections between the two groups. No significant difference was recorded when comparing prevalence of bronchial wall thickening, mucus plugging and centrilobular nodules and signs of emphysema.

Broncho-Alveolar Lavage

All patients underwent bronchoscopy for microbiologic analysis of BALF during diagnostic work-up. BALF cell differential count was available for 21 patients (all with defined GLILD). Mean lymphocytes percentage was 31.42% (SD 24.9), with a median value of 26% (IQ range 18.5–38%) and 15/21 presented a lymphocytosis higher than 20%. When lymphocytes subpopulations analysis was available, mean CD4/CD8 ratio (19 patients) was 2.23 (SD 1.93), median was 1.58 (IQ range 0.53–3.6); 5 patients presented a CD4/CD8 ratio >3.5, as per sarcoidosis diagnostic criteria (18) and 7 > 3.0; in 7 patients ratio was reduced (<1.4). B cell percentage was available for 15 patients, showing a mean 6.82% (SD 5.35), with a median of 6.0% (IQ range 2-10). Five of these patients underwent B cell subpopulations analysis, all showing more than 75% CD21lo B cells.

Logistic Regression Models and ROC Curves

As shown in **Table 5**, DLCO percent of predicted and CD21lo B cells percentage, history of autoimmune cytopenia, and presence of splenomegaly, presented a high power in predicting GLILD.

The final multivariate model including the above-mentioned parameters allowed us to reach a better predictive performance. The joint analysis of these four variables together in a multiple logistic regression model yielded an AUC of 0.98 (95% CI: 0.95-1.0) (**Figure 1**). The corresponding equation is:

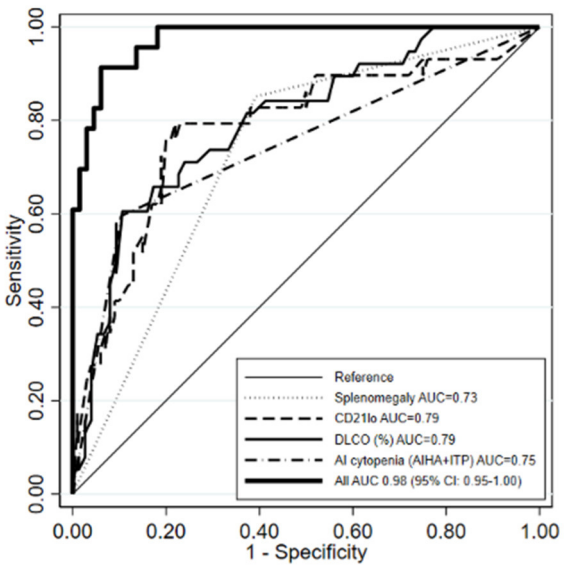
$$\text{Odds (GLILD)} = \exp[-0.530 + (2.136 \times \text{Sp}) + (0.1838 \times \text{CD}) - (0.063 \times \text{DL}) + (3.810 \times \text{AI})]$$

where Sp = splenomegaly (yes = 1), CD = CD21lo (%), DL =DLCO (%) and AI = autoimmune cytopenia (yes = 1). Hence the predicted probability of GD1 can be calculated as: 100 x [odds(GLILD)/[1 + odds(GLILD)].]

TABLE 5 | Univariate logistic regression analysis and area under ROC curve for different possible GLILD predictors.

	GLILD vs. Controls *n*	Odds Ratio (95% C.I.)	*p value*	AUC
IgA at diagnosis (mg/dl)	47 vs. 125	0.97 (0.95–0.99)	0.008	0.65
IgG at diagnosis (mg/dl)	47 vs. 125	0.997 (0.995–0.999)	0.048	0.60
CD21lo B cells %	29 vs. 100	1.099 (1.05–1.15)	<0.001	0.78
FVC (% of predicted)	44 vs. 99	0.96 (0.94–0.98)	<0.001	0.71
DLCO (% of predicted)	38 vs. 75	0.94 (0.91–0.96)	<0.001	0.80
TLC (% of predicted)	34 vs. 64	0.95 (0.92–0.98)	0.001	0.71
AI Cytopenia (ITP, AIHA)	47 vs. 125	12.69 (5.60–28.77)	<0.001	0.75
ITP	47 vs. 125	18.11 (7.23–45.37)	<0.001	0.74
Splenomegaly	47 vs. 125	8.86 (3.68–21.36)	<0.001	0.73

n, number of observations. ITP, AIHA: history of ITP and/or AIHA.



GLILD vs Controls n=23 vs n=66	Odds Ratio (95% C.I.)	p value
CD21lo B cells %	1.20 (1.06-1.36)	0.005
DLCO (% of predicted)	0.94 (0.89-0.99)	0.026
AI Cytopenia (ITP, AIHA)	45.17 (4.76-428.56)	0.001
Splenomegaly	8.47 (1.07-67.20)	0.043

FIGURE 1 | ROC curve of the multiple logistic regression model. The ROC curve of the multiple logistic regression model underlies an AUC of 0.98. Number of observations 89 (23 GLILD and 66 controls). The graph also shows the ROC curves for the logistic regression analysis of the single variables.

When we compared model predictions with actual diagnoses, we observed that, when the probability predicted by the equation was <50%, there were only four subjects with GLILD out of 67 (6.0%); when the predicted probability was 50% or more, the observed frequency was 86.4% (19/22). This means that in order to have a strong indication of the presence of GLILD in a given subject, the probability predicted from the algorithm should be quite high (50% or more).

GLILD and Other uILD Patients

As recapitulated in the previous tables and figures, uILD and GLILD patients did not differ only for the histologic evidence of granuloma. However, uILD patients presented many similarities and few differences when compared to the GLILD group. In terms of demographics, uILD patients appeared to have later CVID onset and a shorter history of disease (**Table 1**). In terms of clinical phenotypes, uILD patients presented a lower prevalence of cytopenia and lymphoproliferation compared to GLILD, but the prevalence was still significantly higher than in controls; the prevalence of bronchiectasis and splenomegaly was similar to GLILD (**Table 2**). When moving to immunologic parameters, uILD patients showed a significant reduction in IgG and IgA levels at CVID diagnosis if compared to controls, similarly to the GLILD group, and as for GLILD required higher dosage of IgRT than controls in order to achieve similar IgG trough levels (**Supplementary Figure 1**). The lower lymphocyte count and higher percentage of CD21lo % of B cells compared to controls were confirmed in uILD as shown for GLILD patients, despite being less significant. uILD patients also showed a significantly lower percentage of circulating CD4+ T cells (**Table 3** and **Supplementary Table 2**).

GLILD patients presented a worse respiratory function if compared to uILD patients, with lower values of all considered parameters and a significant difference, in particular, when considering FVC and DLCO percent of predicted (**Table 4**). However, both DLCO and TLC of uILD patients resulted to be significantly lower than controls.

In conclusion this uILD group, despite presenting a shorter history of disease and a lower prevalence of autoimmune cytopenias, appeared to be quite similar to the GLILD group when considering the main putative predictors of CVID-ILD. This is confirmed by the ROC curve of the multivariate analysis including all CVID-ILD patients, showing an AUC of 0.92 (**Supplementary Figure 2**) when considering the same clinical and immunologic parameters in the GLILD population only, and by the history of GLILD specific

treatment (**Supplementary Table 3**) showing that the prevalence of immune-suppressive treatment was higher in GLILD ($p < 0.01$) but, when specific indication was determined by interstitial lung disease, it was no more significantly different between GLILD and uILD patients (51.0 vs. 26.9%; $p = 0.052$).

DISCUSSION

CVID-ILD represents a relevant clinical issue in the management of CVID patients. Solid data regarding pathogenesis, diagnostic and prognostic markers, as well as treatment strategies are currently lacking. Moreover, different definitions such as CVID-ILD and GLILD are used in literature, whose borders and subsequent clinical implications are not clearly defined. For example, recent studies regarding clinical predictors of CVID-ILD did not routinely distinguish patients according to the presence or absence of a histologic confirmation of GLILD, despite using GLILD as nomenclature, while published retrospective cohorts exploring therapeutic approaches tend to focus on histologically defined ILDs (7, 12–14, 20, 21, 23). At present, retrospective studies on single-center or multicenter cohorts still constitute the main sources of information for Clinicians. To our knowledge, this is the first Italian multicenter study on CVID patients affected by interstitial lung disease (ILD). In our study we aimed to investigate clinical predictors and course of patients with a definite diagnosis of GLILD and those with similar/identical radiologic features not fulfilling the most accredited criteria for GLILD, that we named as undefined ILD (uILD) (9, 20, 29). We first compared the definite GLILD group with a control group of CVID patients without signs of interstitial lung disease.

Using the Chapel classification of CVID main clinical features, we found in the GLILD group an increased frequency of the lymphoproliferation and cytopenia phenotypes and a higher prevalence of clonal lymphoproliferative diseases when pooling together B cell lymphomas and T-LGLL. GLILD patients also showed a higher prevalence of splenomegaly and autoimmunity, mainly due to autoimmune cytopenias, in line with previously published data (14, 20, 21). Differently from what reported by Mannina et al. (21) polyarthritis was not registered at all in our CVID-ILD and controls, as in Versky's cohort. Interestingly, a higher prevalence of bronchiectasis was identified between our GLILD patients, which explains also the more frequent use of antibiotic prophylaxis, compared to previously published data. Low IgA serum levels in CVID have been reported as risk factors for development of bronchiectasis (30). Considering that GLILD patients have lower IgA levels when compared to controls, this could be a plausible explanation for the increased presence of bronchiectasis in our cohort. It does not seem related, instead, to CVID duration, since this was not different between cases and controls.

Immunological evaluation of our cohort of GLILD patients confirmed lower IgG and IgA levels at diagnosis, together with a requirement for a higher dose of IgRT in order to reach IgG trough levels similar to controls. GLILD patients presented lower percentage of switched-memory B cells and marginal zone B cells, as shown by Mannina et al. (21). Finally, they showed a significant increase in the percentage of circulating CD21lo B cells as reported by Hartono et al. (14).

As described in other cohorts, our GLILD patients also had lower lymphocyte counts, with a reduction in CD8+ T cells and an increase in CD4/CD8 ratio when compared to controls. Similar findings were recently reported by Kellner et al. (7) and were associated with increased frequency of pneumonia, herpes viruses and fungal infections. Our study on the other hand was not designed to compare infections rate and type between CVID-ILD and controls. However, we found a higher prevalence of bronchiectasis, smB cells reduction, lower IgG and IgA levels at CVID diagnosis, together with a more frequent use of antibiotic prophylaxis in the GLILD group. It is also to be considered that ILD patients, as in our cohort, might more frequently receive steroids and immune-suppressive drugs both for ILD and associated autoimmune complications (e.g., AI cytopenia) which may also increase the susceptibility to infections (20).

Of note, our CVID-ILD patients presented a significant expansion of CD3CD8CD57+ large T granular lymphocytes, in few patients recognized as T-LGLL; this might be related to splenomegaly/splenectomy, but the same population and T-LGLL itself are known to be related to autoimmune rather than cancer-related manifestations and deserves further investigation (31).

The study of lung function showed in our GLILD cohort lower FEV1%, FVC%, TLC%, and DLCO% compared to controls, with statistically significant differences particularly in FVC%, TLC%, and DLCO%. These data, except for TLC were already reported by Mannina et al. (21) but are quite far from what reported by Hartono et al. (14) We hypothesize that the difference in lung function between ours and other cohorts might rely on different length of CVID history, diagnostic delay, or other population-specific variables such as BMI, coexistence of asthma/COPD and related therapy.

By univariate logistic regression analysis, we explored the performance of the above discussed variables in predicting GLILD diagnosis, and we found presence of splenomegaly and autoimmune cytopenias, IgG and IgA levels at CVID diagnosis, CD21lo B cells percentage, TLC, FVC, and DLCO percent of predicted all presenting low p values. Most of these variables had already been somehow evaluated in previously proposed predictive models for GLILD. We finally defined a predictive model including autoimmune cytopenias, splenomegaly, DLCO percent-of-predicted, and CD21lo B cells percentage, that produced an area under the ROC curve of 0.98. Previously proposed models included either cytopenia, splenomegaly and CD21lo% without any lung function parameter (14) or hypersplenism and FVC% but without any immunologic marker (21). Conversely, our predictive model pools together two clinical variables, CD21lo B cells percentage as immunologic and DLCO% as lung function parameter.

We strongly agree with Mannina et al. (21) on the importance of including a lung-related parameter in a tool that is designed to help diagnosing a systemic disease with a focus on lung interstitium. DLCO and FVC are the key measures in the follow-up and treatment indication of ILDs. DLCO, compared to FEV1,

is less affected by concomitant broncho-active treatment. The sensitivity of HRCT at detecting early signs of ILD is well recognized, as shown by Verbsky et al. (20) but still there is lack of evidence-based data on how and when to treat CVID-ILD patients. Hence, it is reasonable to take into account lung function decline when defining treatment indication, provided that ILD is the actual indication for treatment (20).

On the other hand, it is reasonable to include CD21lo B cells in a predictive model for GLILD, as this subset of B cells has been previously reported to be expanded in CVID patients, expressing pro-inflammatory chemokine receptors predicting the ability of tissue homing like the bronchoalveolar space have the capacity to home to sites of inflammation (32). We indeed reported data on BALF analysis showing that, in all 5 GLILD patients where B cell subpopulations analysis was available, more than 75% of these cells were actually CD21lo B cells. Moreover, in agreement with existing literature, we found a significant BALF lymphocytosis without univocal behavior of CD4/CD8 ratio, and with an increase of B cell percentage in a subgroup of patients.

Broncho-alveolar lavage is routinely used in GLILD work-up for microbiological differential diagnosis. However, BALF findings might also provide data on the different pathogenetic mechanisms and patients' prognosis (18, 33). Thus, we may hypothesize that a more widespread use of BALF analysis and uniformed lymphocyte phenotyping might help to dissect the ongoing lung inflammatory processes (e.g., presence of a CD4+ alveolitis, B cell increase and activation, mediators potentially acting as activity biomarkers) and to potentially define tailored treatments that, at present, are provided only by histologic evaluation.

Finally, as also reported in previous studies, our GLILD and uILD sub-cohorts showed definitely more similarities than differences, as confirmed by the multivariate logistic regression; when we applied our algorithm to the uILD cohort, we identified a subgroup of uILD patients with high probability of GLILD despite the lack of a histologic diagnosis. This raises the question whether the histologic investigation is always mandatory or should be limited to specific cases. Histology is currently the gold standard for GLILD diagnosis. However, we hypothesize that a clinical-radiologic evaluation, in an appropriate multidisciplinary context and with the support of our proposed prediction model (under validation) might be enough for GLILD diagnosis in a proportion of cases, particularly with the aid of genetics and BALF results as possible histologic surrogate. Further studies are needed to confirm our hypothesis. Our study has several limitations, shared with previous study published on this topic, mainly due to the retrospective study design and to the non-univocal definition of CVID-ILD, which is yet an unsolved issue. Despite this, the strengths of this study are the numerous cohorts of GLILD and controls enrolled, the multicentric design and the multidimensional comparison between groups of patients. In conclusion, our findings highlight the strong need for prospective multicenter studies in the complex field of ILD in CVID in order to ameliorate diagnostic tools and prognosis for affected patients.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Comitato Etico delle Province di Treviso e Belluno. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

FC, CM, RS, IQ, and CA conceptualized the study. FC, CM, VL, APl, and RS designed the protocol study. GG, VS, HB, SG, APu, GL, GC, CiM, MC, GT, CaM, SD, MR, AV, and GF recruited patients and collected data. DC and FC did the statistical analysis. NL, SV, and MB performed the radiological analysis. FC, CM, MC, VL, DF, RS, and GS prepared the first draft of the manuscript. All authors reviewed the manuscript before publication.

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SUPPLEMENTARY MATERIAL

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Interstitial Lung Disease in Common Variable Immunodeficiency

Joao Pedro Lopes[1,2], *Hsi-en Ho*[2] *and Charlotte Cunningham-Rundles*[2]*

[1] Division of Allergy, Immunology and Rheumatology, Department of Pediatrics, UH University Hospitals Rainbow Babies and Children, Cleveland, OH, United States, [2] Division of Clinical Immunology, Department of Medicine, Icahn School of Medicine at Mount Sinai, New York, NY, United States

Interstitial lung disease (ILD) is a common complication in patients with common variable immunodeficiency (CVID) and often associated with other features, such as bronchiectasis and autoimmunity. As the ILD term encompasses different acute and chronic pulmonary conditions, the diagnosis is commonly made based on imaging features; histopathology is less frequently available. From a cohort of 637 patients with CVID followed at our center over 4 decades, we reviewed the data for 46 subjects (30 females, 16 males) who had lung biopsies with proven ILD. They had a median age at CVID diagnosis of 26 years old, with a median IgG level at diagnosis of 285.0 mg/dL with average isotype switched memory B cells of 0.5%. Lung biopsy pathology revealed granulomas in 25 patients (54.4%), lymphoid interstitial pneumonia in 13 patients (28.3%), lymphoid hyperplasia not otherwise specified in 7 patients (15.2%), cryptogenic organizing pneumonia in 7 patients (15.2%), follicular bronchitis in 4 patients (8.7%), and predominance of pulmonary fibrosis in 4 patients (8.7%). Autoimmune manifestations were common and were present in 28 (60.9%) patients. Nine patients (19.6%) died, with a median age at death of 49-years-old. Lung transplant was done in 3 of these patients (6.5%) who are no longer alive. These analyses reveal the high burden of this complication, with almost one-fifth of the group deceased in this period. Further understanding of the causes of the development and progression of ILD in CVID patients is required to define the best management for this patient population.

Keywords: common variable immune deficiency (CVID), interstitial lung disease (ILD), autoimmunity, lung transplant, cytopenia, malignancy, lymphoma

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***Correspondence:**
Charlotte Cunningham-Rundles charlote.cunningham-rundles@mssm.edu

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INTRODUCTION

Common variable immunodeficiency (CVID) is the most prevalent form of clinically-recognized primary immunodeficiency, characterized by low serum IgG levels, usually a low IgA, and often a low IgM, reduced or absent antibody responses to disease or immunizations. This defect leads to recurrent infections, with particular emphasis on the sinorespiratory tract (1–6). CVID is also commonly associated with inflammatory complications, leading to chronic lung disease, generalized lymphoid hypertrophy, splenomegaly, gastrointestinal disease, and cytopenias, amongst other inflammatory manifestations (7–11). Interstitial lung disease (ILD) is a term that encompasses a group of different acute and chronic pulmonary conditions with common clinical and physiological characteristics. This condition is a common complication in patients with CVID. The diagnosis of ILD is commonly made based on clinical presentation and includes

characteristic imaging features. For those in whom a biopsy was performed, histology provides further confirmation of the diagnosis, along with the individual pathologic features (12–14).

Chronic lung disease, including ILD, is often associated with other inflammatory features, such as lymphoid hyperplasia and autoimmunity. When present, the lung damage is associated with shortened survival; this has been noted as the leading cause of death in some CVID cohorts (5, 7, 9, 15–22). Previous publications have addressed the frequency of clinically diagnosed ILD in CVID, noted in the 10–20% range (15–18, 23). Bates et al. on a cohort of 69 CVID patients showed reduced survival for ILD vs. non-ILD patients (17). In this study, the ILD diagnosis was associated with a propensity for T cell lymphopenia, splenomegaly, and restrictive pulmonary physiology (17). Relative lymphopenia was also noted in data on a cohort from the USIDNET (United States Immunodeficiency Network) Registry; here, Kellner et al. analyzed data from 1,518 CVID patients, of whom 138 patients (9.1%) had an ILD diagnosis. These patients had lower CD3, CD4, and CD8 T cell counts than patients without ILD, suggesting an increased risk of complications related to these abnormalities (18).

While the pathogenesis of ILD in a significant number of CVID patients remains unclear, genetic defects, and T cell and B cell dysregulation have been associated with progression. As suggested by Weinberger et al. based on comparing patients with X-linked agammaglobulinemia (XLA) to CVID patients in the USIDNET Registry, lack of antibody alone would not appear to be the leading cause of ILD, as a higher frequency of ILD, as well as of respiratory infections and asthma, was described in CVID when compared to patients with XLA (24).

In the absence of a consensus in terms of the best therapeutic approach for CVID patients with ILD (25), several therapeutic options have been discussed with an attempt to control, even if not to reverse, the progression of ILD in CVID patients (20). These include rituximab, corticosteroids, and a number of other immunosuppressive agents (26–28).

Here we review data on a group of patients in our New York CVID cohort who had biopsy-proven ILD, examining their clinical, laboratory, radiologic, histopathological, and functional data. We also aimed to review the existing data on the spectrum of ILD in CVID, to put our findings in perspective of the joint efforts by other groups to better understand this presentation's pathophysiology and potential avenues for better prevention and treatment in the near future.

MATERIALS AND METHODS

Patients

A cohort of 637 subjects with CVID (337 females and 285 males) were seen at Mount Sinai Medical Center from 1986 through the present. The first part of the cohort was previously seen at Memorial Sloan-Kettering Cancer Center (1974–1986); subsequently, these subjects were seen at Mount Sinai Medical Center. The diagnosis of CVID was made by standard criteria, including reduced serum IgG, IgA, and/or IgM, by at least 2 SDs below the mean for age, with poor or absent antibody production to both protein and carbohydrate vaccines and exclusion of other causes of hypogammaglobulinemia. Subjects under age 4 years without continued follow-up and subjects with lymphoid cancer diagnosed within 2 years after the diagnosis of CVID were excluded. For the 46 patients with biopsy-proven ILD, medical, radiologic immunologic, and pathology data were reviewed for this report.

Immunologic Parameters

Enumeration of T and B cells, CD4, and CD8 T cells, and $IgM^{-}IgD^{-}CD27^{+}$ isotype switched memory B cells as a proportion of total B cells were determined.

Data

Data was abstracted in Microsoft Excel and analyzed in IBM SPSS Statistics. All studies were undertaken with the consent of the Mount Sinai Medical Center Institutional Review Board.

RESULTS

Demographics and Immune Phenotypes

Forty-six patients with biopsy-confirmed ILD and for whom pathology reports were available, included 30 females and 16 males. Two patients were African American; the rest were Caucasian. The age at CVID diagnosis was 26 years (range 1.0–66.0 years old), with lung symptoms appearing, as noted in the chart, at a median age of 29 years (range 1.0–59.0 years old). Immunoglobulin replacement was started later, at a median age of 32.5 years (**Table 1**). Baseline immunoglobulins, IgG, IgA, and IgM, are noted in **Table 2**, with the values presented in the Table for this group of 46 patients with CVID and ILD, similar to 500 other CVID subjects in this cohort with no known ILD (IgG = 246+/−221 mg/dL; IgA = 7.0+/−30.4 mg/dL, and IgM=20+/−166.4 mg/dL) (Mann-Whitney test). Absolute CD3, CD4, CD8 T cells, and CD19 B lymphocyte numbers were overall within normal limits but with wide variation. The percent of isotype switched memory B cells were low, as characteristic of CVID subjects (**Table 2**).

Pathology

Lung biopsy pathology revealed granulomatous infiltrates in 25 of the 46 patients (54.3%), lymphoid interstitial pneumonia in 13 (28.3%), lymphoid hyperplasia not otherwise specified in 7 (15.2%), cryptogenic organizing pneumonia in 7 (15.2%), follicular bronchitis in 4 (8.7%), and predominance of pulmonary fibrosis in 4 patients (8.7%). Combinations of these pathologic findings were found in several subjects (**Table 3**). **Figure 1** contains the lung biopsy of one of the ILD/CVID patients in this group, demonstrating the presence of a granulomatous lesion

TABLE 1 | Demographic information of CVID patients with ILD included in the study.

Age (46 subjects, 30 females, 16 males)	
CVID diagnosis (years-old; median, SD)	26.0 (17.8)
Onset lung symptoms (years-old, median, SD)	29.8 (16.5)
Ig replacement (years-old, median, SD)	32.5 (17.5)

TABLE 2 | Immunologic laboratory data of CVID patients with ILD.

Immunoglobulins	**(Median +/– SD)**
Baseline IgG (normal 700–1,600 mg/dL)	**285.0** mg/dL (232.0)
Baseline IgA (normal 90–386 mg/dL)	**6.5** mg/dL (11.2)
Baseline IgM (normal 20–172 mg/dL)	25.5 mg/dL (59.0)
Lymphocytes **(average count, SD)**	**(median +/– SD)**
ABS CD3 T cells (normal 575–2,237/μL)	898.0 (714.8) range 278–4232
ABS CD4 T cells (normal 325–1,472/μL)	555.0 (476.1) range 203–2828
ABS CD8 T cells (normal 109–897/μL)	366.0 (287.6) range 62–1404
ABS B cells (normal 12–645/mm3)	83.4 (128.7) range 0–512
Isotype switched CD27+B cells (% of B cells] (normal 10–22.2%)	**0.5%** (1.4%) range 0–5.8%

Values bold when outside the normal reference range.

TABLE 3 | Lung pathology encountered in the CVID patients with ILD.

	Number	**Percent**
Granulomas	17	37
LIP	7	15.2
COP	4	8.7
Fibrosis	3	6.5
LIP, granulomas	3	6.5
Lymphoid hyperplasia	3	6.5
LIP, follicular bronchiolitis	2	4.3
COP, fibrosis	1	2.2
COP, granulomas	1	2.2
Follicular bronchiolitis, lymphoid hyperplasia, granulomas	1	2.2
LIP, COP, granulomas	1	2.2
Lymphoid hyperplasia	1	2.2
Lymphoid hyperplasia, granulomas	1	2.2
Lymphoid hyperplasia, granulomas, follicular bronchiolitis	1	2.2
Total	46	100

COP, Cryptogenic organizing pneumonia; LIP, lymphocytic interstitial pneumonia.

with lymphocytic infiltration. Bronchiectasis was concomitantly described in 8 (17.4%) patients.

Lung Functions

Results available for 28 patients (60.9%) revealed the group to have an average FEV1/FVC ratio of 0.85 (standard deviation of 0.11; normal ratio above 0.75), with an average FEV1 of 0.71 (standard deviation of 0.17; normal FEV1 above 0.80) and FVC of 0.72 (standard deviation of 0.18; normal FVC above 0.80). The average TLC was 0.78 (standard deviation of 0.14; normal TLC above 0.80) and the average DLCO was 60.8% of predicted (standard deviation 22.3%, range 16.0 to 109.0%; normal DLCO above 75.0% of predicted).

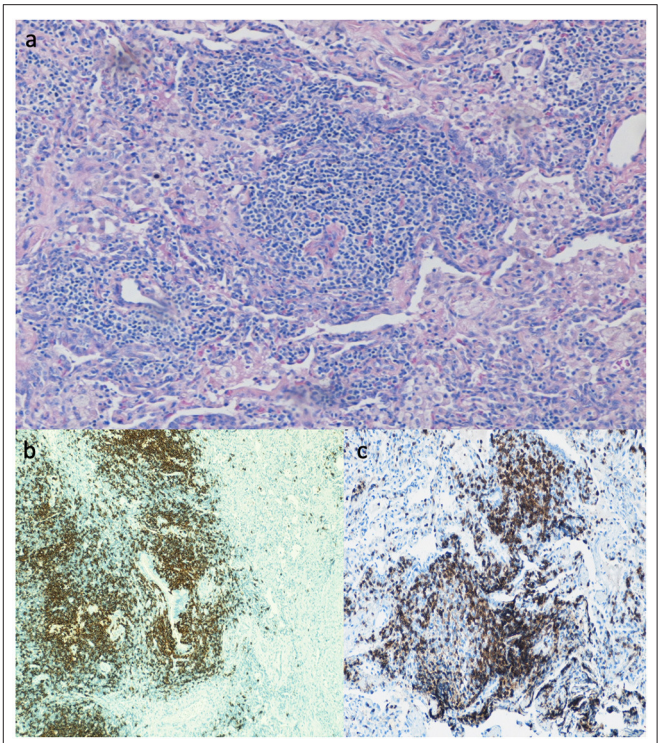

FIGURE 1 | Lung biopsy of CVID patient with ILD, showing the presence of a granuloma lesion, with presence of lymphocytes on H&E staining **(a)**, immunohistochemistry for CD20 **(b)**, and for CD3 cells **(c)**. Magnification of images is 40x.

Radiologic Studies

Chest x-rays were available for 15 patients (32.6%), describing the presence of nodular opacities in 12 patients (80.0%) reticular infiltrates in 6 patients (40.0%), and fibrosis in 2 patients (13.3%). Computerized tomography scans of the chest were available for 32 patients (69.6%). Findings were notable for the presence of nodules in 30 patients (93.8%), mediastinal lymphadenopathy in 21 patients (65.6%), ground-glass appearance in 12 patients (37.5%), diffuse consolidation in 4 patients (12.5%), granulomas in 2 patients (6.3%), and fibrosis in 1 patient (3.1%). Examples of radiologic findings are shown in **Figure 2** (chest x-ray) and **Figure 3** (chest CT scan).

Clinical Features

Autoimmune manifestations other than in the lung were present in 28 (60.9%) patients, with 12 of those patients (26.1%) having more than one autoimmune manifestation. Cytopenias were a common manifestation: immune thrombocytopenic purpura (ITP) in 18 patients (39.1%), autoimmune hemolytic anemia (AIHA) in 9 patients (19.6%), autoimmune neutropenia in 6 patients (13.0%), pancytopenia in 3 patients (6.5%), and red blood cell aplasia in 2 patients (4.3%). Many had more than one of these conditions, most commonly, AIHA and ITP. Other conditions included uveitis, severe aphthous ulcers, primary biliary cholangitis, and rheumatoid arthritis in one patient each. Twenty nine patients (63.0%) were observed to have lymphadenopathy, and the same number to have splenomegaly. Splenectomy had been done in 10 of these patients (21.7%).

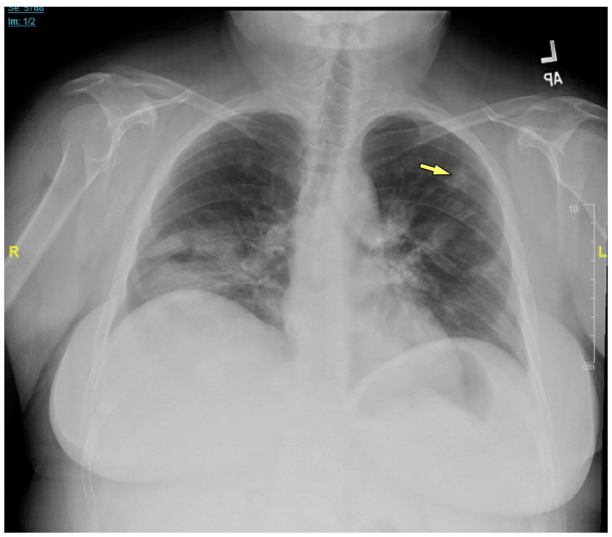

FIGURE 2 | Chest x-ray of CVID/ILD patient demonstrating presence of 1.6 cm lesion in the left upper lobe (yellow arrow), as well as patchy densities in the mid to lower lung fields.

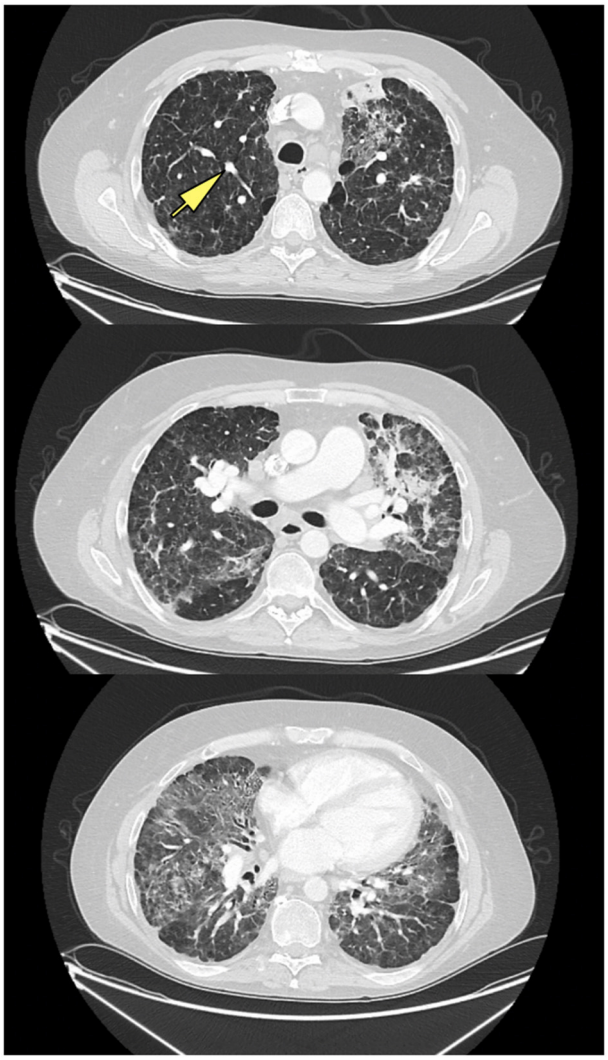

FIGURE 3 | CT scan of ILD/CVID patient, with upper, middle, and lower lung zones, demonstrating mid to lower lung zone predominant ground glass opacities, within a bronchovascular distribution, with associated volume loss, with left upper lobe consolidation in association with air bronchograms, likely pneumonia, new solid nodular opacity (12 × 7 mm) (yellow arrow) in the right upper lobe, as well as bilateral hilar lymphadenopathy.

Nodular regenerative hyperplasia of the liver was noted in 7 patients (15.2%). Five of these patients (10.9%) developed a malignancy, with 4 (8.7%) developing a lymphoma. One other patient had ovarian cancer.

Unusual infections were identified in several of these patients: 5 patients (10.9%) had herpes zoster (caused by the varicella zoster virus), 1 patient (2.2%) had atypical mycobacteria lung infection, 1 patient (2.2%) had measles encephalitis, 1 patient (2.2%) had metapneumovirus infection, and 1 patient (2.2%) had *Pseudomonas* otitis complicated by *Pseudomonas* bacteremia.

Genetics

A gene mutation associated with or contributing to the patient's CVID phenotype was identified in 10 of the 31 subjects available for testing by whole-exome sequencing (32.3%); 3 patients (9.7%) had a *CTLA4* mutation, 2 patients (6.5%) had an *NFKB1* mutation, 2 patients (6.5%) had either one or two TACI (*TNFRSF13B*) mutations, and 1 each had a *STAT3* mutation, *KMT2D* mutation, or a *PIK3CD* mutation (3.2%).

Treatment and Outcomes

Treatment modalities used in these subjects are outlined in **Table 4**. Seven patients (15.2%) required chronic oxygen supplementation, and 5 patients (10.9%) were diagnosed with pulmonary hypertension. Lung transplant had been done in 3 of the patients described here (6.5%); none are currently surviving (**Table 5**). Overall, 9 of these patients (19.6%) have died, with a median age of death of 49.0 years-old (range 27.0–70.0 years-old, standard deviation of 15.1 years).

DISCUSSION

We describe 46 patients with biopsy-characterized ILD in our cohort of 637 CVID patients, 7.2% of the cohort. As recently published, based on both radiologic studies and pathology, the overall frequency of ILD in our CVID cohort is 10.4% (15), similar to other reports in which the incidence ranges from 10 to 20% in CVID (16, 18, 23, 29). In this report, we focus on the subjects for whom a biopsy had been done to provide further pathology.

A study by Patel et al. on data from the Oxford Primary Immune Deficiencies Database evaluated lung biopsies from 16 CVID patients, recognizing the presence of lymphocytic infiltrations as the most common pattern. In the Oxford report, 5 of these patients were also evaluated with immuno-markers, showing T cell infiltrates in 4 patients and B cell infiltrates in one other individual (30). In contrast, analysis of the lung biopsy results in our group demonstrated granulomatous infiltration in more than half of our patients. The commonly used term of granulomatous-lymphocytic interstitial lung disease (GLILD)

TABLE 4 | Treatment modalities used in the CVID patients with ILD in the study.

Corticosteroids	23 patients (50.0%)
Rituximab	16 patients (34.8%)
Mycophenolate mofetil	5 patients (10.9%)
Azathioprine	4 patients (8.7%)
Mercaptopurine	2 patients (4.3%)
Hydroxychloroquine	2 patients (4.3%)
Abatacept	1 patient (2.2%)
Sirolimus	1 patient (2.2%)
Cyclosporine	1 patient (2.2%)

TABLE 5 | Lung transplant characteristics in this cohort.

	Patient 1	Patient 2	Patient 3
Year born	1959	1963	1949
Lung pathology	Chronic obstructive pulmonary disease	Pulmonary fibrosis predominates with granuloma	ILD (granuloma and lymphoid infiltrate), bronchiectasis
CVID-associated comorbidities	Enteropathy	Liver disease	None
Transplant procedure	Lung and heart	Lung	Lung
Year of transplant procedure (age)	1983 (age 34)	1997 (age 34)	2018 (age 70)
Outcome	Died of chronic rejection after 5 years	Operative complications, died of hyperacute rejection within a week	Died of acute rejection after 8 months; CMV infection

can be applied to these subjects. Lymphoid infiltrations were the second most prevalent condition, found in 20 patients.

Almost all patients had a description of numerous lung nodules from the radiologic perspective, and nearly two-thirds had mediastinal lymphadenopathy. More than one third had areas with ground-glass appearance. Only 2 patients had "granulomas" suggested on their CT report. As more than half of the patients had granulomas present in biopsies, it is clear that, from the CT perspective, this form of pathology would not be clarified by radiologic observations. As previously suggested (31), it is important not only to recognize specific CT patterns of ILD but also early lung abnormalities at a subclinical level. Not surprisingly, patients had impaired lung functions with reduced FEV1, FVC, TLC, and DLCO, all in line with a restrictive disease pattern previously described in similar cohorts (17).

In previous studies, B cell dysregulation has been associated with progression of ILD. Maglione et al. analyzed CVID patients with ILD treated with rituximab, noting that recurrence of lung disease was associated with an increase of B cell-activating factor (BAFF) in the peripheral blood; this could potentially lead to the B cell hyperplasia in the lung, with the development of germinal centers as one driver of lung damage in these patients (32). In the same paper, progression of ILD, as well as ILD recurrence post-rituximab, were also seen to be associated with increasingly elevated serum IgM, potentially a reflection of the increasing hyperplasia of local pulmonary B cell follicles (32). However, for the 46 biopsied subjects examined here, serum IgM was not different from 500 other subjects in this cohort without confirmed ILD.

In previous ILD studies, patients have commonly been noted to have many additional inflammatory complications (33). In our group, splenomegaly and lymphadenopathy were present in nearly two-thirds of the patients, and more than half had had cytopenias (mostly ITP, but also AIHA or neutropenia). In one cohort of 105 adult CVID patients, more patients had splenomegaly (74.0%) and lymphadenopathy (63%) than non-ILD patients (16). Maglione et al. also reviewed CT imaging from CVID patients with pulmonary disease; here, while the presence of bronchiectasis was associated with a higher number of infections, imaging patterns of ILD were more frequently associated with autoimmunity and lymphoproliferation (21). The high frequency of splenomegaly and the history of cytopenias were also highlighted as potential predictors of granulomatous lymphocytic interstitial lung disease (GLILD) by Hartono et al. in a 2017 study (33). In our cohort, splenectomy had been performed in more than one-fifth of these patients for one or more of these cytopenias and/or hypersplenism. Noteworthy as well is that nodular regenerative hyperplasia of the liver, another inflammatory condition of unclear etiology, was documented in 7 of these patients (15.2%).

CVID patients are also known to have increased malignancy rates, particularly lymphoma, described with a rate of 1.6–8.2% of CVID patients, depending on the cohort (9, 19, 34). In this group, the rate was 8.7%, a remarkable reminder of the higher risk for lymphoma in this particular patient group and the importance of appropriate surveillance.

As for treatment, rituximab, a monoclonal antibody targeting CD20, with the goal of B cell depletion, has been successfully used in this patient population, as monotherapy or in combination with other immune suppressants (26, 32, 35, 36). Chase et al. examined combination therapy with rituximab and azathioprine in 7 patients, noting improvement in both pulmonary function and CT abnormalities, without significant treatment side effects (27). Corticosteroids have been one of the mainstays of ILD treatment (28), but as is well-documented, their long-term use is associated with side effects, some of them potentially severe (37). Other immunosuppressive medications, such as mercaptopurine, cyclosporine, hydroxychloroquine, mycophenolate mofetil, or abatacept, have been used, with variable success (38–40). Our group of patients had varying use of different immunosuppressive agents, with half of the group having documentation of corticosteroid use at some point and more than one third having received rituximab, but other agents such as mycophenolate mofetil or azathioprine were also used in this population. Additional data on the response of ILD to different agents will be necessary to, if not reaching a consensus, at least define the best available therapies to contain or reverse the progression of lung disease. More knowledge on the genetics and/or pathogenesis for each patient, may allow some ability to tailor these therapies more individually.

That almost one-fifth of the patients discussed here died with a median age of death of 49 years-old is a striking reminder of the shortened life expectation for CVID patients with ILD. This is also highlighted by the 7 patients requiring chronic oxygen and the 5 diagnosed with pulmonary hypertension

requiring additional therapies. Lung transplantation has been done increasingly for several end-stage lung diseases, and post-transplant survival has improved in the last decades (41). The three patients in our group of 46 with biopsy-proven ILD who underwent lung transplant died. Parenthetically, out of the 637 CVID patients followed in our center, a total of 8 patients have now undergone lung transplant (three of these are part of the 46 patients in this cohort described in **Table 5**). Only one of these 8 patients submitted to lung transplant is now alive. It remains unclear for which CVID patients with end-stage respiratory disease this would be a viable option.

As our cohort spans almost 50 years of follow-up, only 31 of the 46 patients had a genetic evaluation, but in these, 10 had genes now identified as leading to or associated with this immune defect. In some cases (*CTLA4, STAT3*), these data may help in suggesting more targeted therapies for ILD (abatacept, or tocilizumab as an anti–IL-6 receptor mAb). The increasing use of genetic analysis has helped to better understand and define the CVID syndrome (42–45) and, hopefully, will lead to a better understanding of the pathogenesis and/or suggest new therapies.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Icahn School of Medicine at Mount Sinai Institutional Review Board. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

All authors participated in the data collection, data analysis, manuscript writing, and manuscript review of the research data here presented.

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Conflict of Interest: The authors declare that research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Treatment Strategies for GLILD in Common Variable Immunodeficiency: A Systematic Review

Olivia A. C. Lamers[1†], Bas M. Smits[1,2†], Helen Louisa Leavis[2], Godelieve J. de Bree[3], Charlotte Cunningham-Rundles[4], Virgil A. S. H. Dalm[5], Hsi-en Ho[4], John R. Hurst[6], Hanna IJspeert[5], Sabine M. P. J. Prevaes[7], Alex Robinson[6], Astrid C. van Stigt[5], Suzanne Terheggen-Lagro[8], Annick A. J. M. van de Ven[9], Klaus Warnatz[10,11], Janneke H. H. M. van de Wijgert[12] and Joris van Montfrans[1]*

[1] Department of Pediatric Immunology and Rheumatology, Wilhelmina Children's Hospital, Utrecht, Netherlands, [2] Department of Immunology and Rheumatology, University Medical Center Utrecht, Utrecht, Netherlands, [3] Department of Internal Medicine, Amsterdam University Medical Center, Amsterdam, Netherlands, [4] Department of Medicine, Division of Clinical Immunology and Department of Pediatrics, Mount Sinai Hospital, New York, NY, United States, [5] Department of Internal Medicine, Division of Clinical Immunology and Department of Immunology, Erasmus University Medical Center Rotterdam, Rotterdam, Netherlands, [6] UCL Respiratory, University College London, London, United Kingdom, [7] Wilhelmina Children's Hospital, Department of Pediatric Pulmonology, Utrecht, Netherlands, [8] Department of Pediatric Pulmonology, Amsterdam University Medical Center, Amsterdam, Netherlands, [9] Departments of Rheumatology and Clinical Immunology, Internal Medicine and Allergology, University Medical Center Groningen, Groningen, Netherlands, [10] Department of Immunology, Universitätsklinikum Freiburg, Freiburg, Germany, [11] Department of Rheumatology and Clinical Immunology, Division of Immunodeficiency, Medical Center - University of Freiburg, Faculty of Medicine, Freiburg, Germany, [12] Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht University, Utrecht, Netherlands

Introduction: Besides recurrent infections, a proportion of patients with Common Variable Immunodeficiency Disorders (CVID) may suffer from immune dysregulation such as granulomatous-lymphocytic interstitial lung disease (GLILD). The optimal treatment of this complication is currently unknown. Experienced-based expert opinions have been produced, but a systematic review of published treatment studies is lacking.

Goals: To summarize and synthesize the published literature on the efficacy of treatments for GLILD in CVID.

Methods: We performed a systematic review using the PRISMA guidelines. Papers describing treatment and outcomes in CVID patients with radiographic and/or histologic evidence of GLILD were included. Treatment regimens and outcomes of treatment were summarized.

Results: 6124 papers were identified and 42, reporting information about 233 patients in total, were included for review. These papers described case series or small, uncontrolled studies of monotherapy with glucocorticoids or other immunosuppressants, rituximab monotherapy or rituximab plus azathioprine, abatacept, or hematopoietic stem cell transplantation (HSCT). Treatment response rates varied widely. Cross-study comparisons were complicated because different treatment regimens, follow-up

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University Hospital La Paz, Spain

***Correspondence:**
Joris van Montfrans
J.vanMontfrans@umcutrecht.nl

[†]These authors have contributed equally to this work

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periods, and outcome measures were used. There was a trend towards more frequent GLILD relapses in patients treated with corticosteroid monotherapy when compared to rituximab-containing treatment regimens based on qualitative endpoints. HSCT is a promising alternative to pharmacological treatment of GLILD, because it has the potential to not only contain symptoms, but also to resolve the underlying pathology. However, mortality, especially among immunocompromised patients, is high.

Conclusions: We could not draw definitive conclusions regarding optimal pharmacological treatment for GLILD in CVID from the current literature since quantitative, well-controlled evidence was lacking. While HSCT might be considered a treatment option for GLILD in CVID, the risks related to the procedure are high. Our findings highlight the need for further research with uniform, objective and quantifiable endpoints. This should include international registries with standardized data collection including regular pulmonary function tests (with carbon monoxide-diffusion), uniform high-resolution chest CT radiographic scoring, and uniform treatment regimens, to facilitate comparison of treatment outcomes and ultimately randomized clinical trials.

Keywords: systematic review, immunodeficiency, common variable immunodeficiency, CVID, granulomatous lymphocytic interstitial lung disease, GLILD, treatment

INTRODUCTION

Common variable immunodeficiency disorders (CVID) are the most common symptomatic primary immunodeficiencies, with an estimated incidence between 1:10.000 and 1:50.000 (1). Patients typically suffer from recurrent respiratory tract infections, such as bronchitis, sinusitis, otitis media and pneumonia. Moreover, they are often affected by immune dysregulation, a term which encompasses auto-immune manifestations, auto-inflammatory disease and lymphoproliferation, and by malignancy (2). Infection risk in CVID can be minimized by means of antimicrobial prophylaxis and immunoglobulin replacement therapy (IgRT). In contrast, immune dysregulation is much more difficult to prevent and treat, and remains a major cause of morbidity and mortality (3–6).

Granulomatous lymphocytic interstitial lung disease (GLILD) is one of the complications of CVID and is considered the pulmonary manifestation of multi-system immune dysregulation. GLILD occurs in approximately 10-20% of patients with CVID and was reported to be responsible for a reduction in life expectancy of more than 50% after diagnosis in adult patients, from a median of 28.9 to 13.7 years (6, 7). GLILD may be asymptomatic, or may present with non-specific symptoms such as cough and dyspnea on exertion (4). Small or large nodules, consolidations and ground glass abnormalities in the lower regions of the lung on high-resolution CT-scan are highly suggestive of GLILD (8). The diagnosis can be confirmed by biopsy (via video-assisted thoracoscopic surgery, transbronchial or percutaneous intervention) and FDG-PET-CT may be used for the identification of active inflammatory lesions elsewhere (4, 9). The combination of routine chest CT-scans and pulmonary function tests, including specifically diffusing capacity of carbon monoxide, should be used to identify GLILD in CVID and monitor disease progression (9).

The etiology of GLILD is still poorly understood. Maglione and colleagues pointed out that patients with X-linked agammaglobulinemia (XLA) have severe antibody deficiency that is even more pronounced than CVID but only rarely develop GLILD (10). Patients with XLA lack mature B-cells, whereas patients with CVID have peripheral B-cells, although often with impaired function, suggesting that B-lymphocytes may play a causative role in GLILD development. Indeed, lymphocytic (but not the granulomatous) progression has been associated with an increased production of B-cell activating factor (BAFF), which in turn leads to activation of the anti-apoptotic factor Bcl-2, thereby promoting B-cell survival as well as an increase of IgM producing CD21 low B-cells (10). Unger et al. linked the expansion of CD21low B-cells with disproportionally high numbers of Th1 cells and increased interferon-γ production, probably reflecting the aberrant combined T-B interaction in the pathogenesis of interstitial lung disease in CVID (11). It has also been suggested that viral infections may trigger GLILD, as Wheat et al. identified a correlation between human herpesvirus 8 (HHV8) infection and the disease (12). However, since the publication of the original article describing this correlation, no further evidence has been provided for this hypothesis. Finally, an association between interstitial lung disease and an increased relative abundance of Streptococcus in the oropharyngeal microbiome in CVID was recently identified (13).

The treatment of GLILD mostly consists of immunosuppressive medication, in addition to IgRT and other supportive measures such as physiotherapy. According to the British Lung Foundation/United Kingdom Primary Immunodeficiency Network Consensus Statement, glucocorticoids are the first line of therapy for GLILD (9). Most clinicians agree that azathioprine, mycophenolate mofetil (MMF) and rituximab are second-line choices when glucocorticoids are not effective or when attempting to spare their use (9). Although

alternative medication may also be prescribed, there is no consensus about the use of other biologic therapies or disease-modifying anti-rheumatic drugs (DMARDs) (9).

Current GLILD treatment guidelines are based on expert opinion rather than on robust scientific evidence. An objective review of the existing evidence is needed to minimize potential biases associated with expert opinion, and to identify knowledge gaps. Therefore, our aim was to systematically review the existing literature on treatment of GLILD in CVID patients. To the best of our knowledge, this is the first systematic review on that topic.

METHODS

We searched PubMed and EMBASE for publications on treatment of GLILD in CVID patients (last search on March 27th 2020, see **Appendix for Search String**). Articles describing patients with CVID and GLILD who were treated with pharmacological therapy and/or a hematopoietic stem cell transplantation (HSCT) were included. Improvement of disease activity parameters (symptoms, pulmonary function tests and radiological findings) and mortality served as outcomes.

We focused our search on patients with CVID and GLILD. Studies describing patients with monogenetic diseases causing a CVID-like phenotype (such as CTLA-4 haploinsufficiency and LRBA deficiency) were included.

The consensus GLILD definition of the British Lung Foundation/United Kingdom Primary Immunodeficiency Network was used: "GLILD is a distinct clinic-radio-pathological interstitial lung disease occurring in patients with CVID, associated with a lymphocytic infiltrate and/or granuloma in the lung, and in whom other conditions have been considered and where possible excluded" (9). Only articles that reported radiological findings on a CT-scan or histological analysis of biopsies compliant with this definition of GLILD were included.

All non-English articles were excluded for purposes of practicality. Conference abstracts, while read and taken into consideration, were excluded from the review as they were not peer-reviewed.

Two independent investigators (O.L. and B.S.) selected articles on the basis of title and abstract. Blinding of the investigators was achieved by inserting all articles in a common online database (Rayyan), which has a blinding feature and allows each researcher to select articles independently of the other. Ultimately, the selection of articles of each researcher was compared to the other. If there were any selection discrepancies, the articles were discussed until a unanimous decision about in- or exclusion could be made. Data were extracted from the eligible full-text articles using a standardized data extraction sheet. The extracted data were summarized descriptively and reported in tables. We could not conduct meta-analyses because the selected articles contained insufficient quantitative data.

If the use of multiple treatment regimens in one patient was reported, the effect of the treatment regimens was evaluated separately. When escalation or switching of treatment was deemed necessary by the authors, the previous regimen was deemed insufficient. To evaluate the effect of treatment regimens, both qualitative and quantitative assessments of GLILD activity were analyzed. Descriptive improvement of pulmonary function tests, radiological findings and symptoms (e.g. "shortness of breath", "coughing") were used for the qualitative evaluation of disease activity. Significant improvement was defined as a relapse-free improvement of at least one of these parameters and no deterioration of the other parameters. Pre- and post-treatment pulmonary function test results were used for the quantitative evaluation of disease activity, and significant improvement of pulmonary function was here defined as a 10% increase in at least one pulmonary function test parameter.

Overall risk of bias of each study was assessed by means of a self-designed tool based on the PRISMA guidelines (14). This tool took into account the quality of the studies (based on the number of patients and controls, and on descriptions of outcomes, medication dosages and follow-up procedures) and possible confounders (smoking, age, comorbidity, and results of genetic testing). Each study was assigned a rating for each of these categories, 'good' (+) if the highest quality standard was attained with clear quantitative outcomes, 'intermediate' (+/-) if some information was reported but quantitative measures were lacking, and 'insufficient' (-) if the information was not reported at all. The overall risk of bias was determined as follows: 'high risk of bias' if the study had four or more insufficient or eight or more intermediate judgments; 'intermediate risk of bias' if the study was marked insufficient on two to four items or intermediate on four to eight items; and 'low risk of bias' if the study had only one insufficient judgment or a maximum of three intermediate judgments.

The level of evidence for each study and the degree of recommendation in clinical practice were determined following the criteria formulated by the Centre for Evidence Based Medicine (15).

RESULTS

The search identified 6124 articles on PubMed and EMBASE and seven additional papers *via* snowballing (**Figure 1**). After removal of duplicates, 5304 articles were screened, 65 full-text papers were read, and 42 articles were deemed eligible. 233 patients were described in total. The findings are summarized below, sorted by treatment modality. Qualitative and quantitative lung function findings are shown in **Figure 2**.

There were three papers describing GLILD in patients with B lymphocyte related primary antibody deficiency other than CVID (such as IgA or IgG subclass deficiency, or selective antibody deficiency for polysaccharide antigens). These articles are listed in the **Supplementary Material** (**Table S1**).

Glucocorticoids

Glucocorticoids have been identified as the first line treatment for GLILD by the British Lung Foundation/United Kingdom Primary Immunodeficiency Network (2017) (9).

Six articles specifically reported on the use of glucocorticoids for the treatment of GLILD in patients with CVID, as shown in **Table 1**. The first report dates back to 1982 and describes the case of a woman who was treated with high-dose prednisone for six weeks. Symptoms initially subsided but relapsed when the medication was tapered (18). Ten additional studies included glucocorticoid treatment as one of several therapies (**Tables 2** and **3**). Five of these reported no effect of glucocorticoids (26, 27, 31, 36–38), one reported relapse after initial remission (29) and four reported treatment success (16, 17, 20, 21). The article by Kanathur et al. is particularly interesting as it describes a case in which glucocorticoids initially failed to have any effect at all but

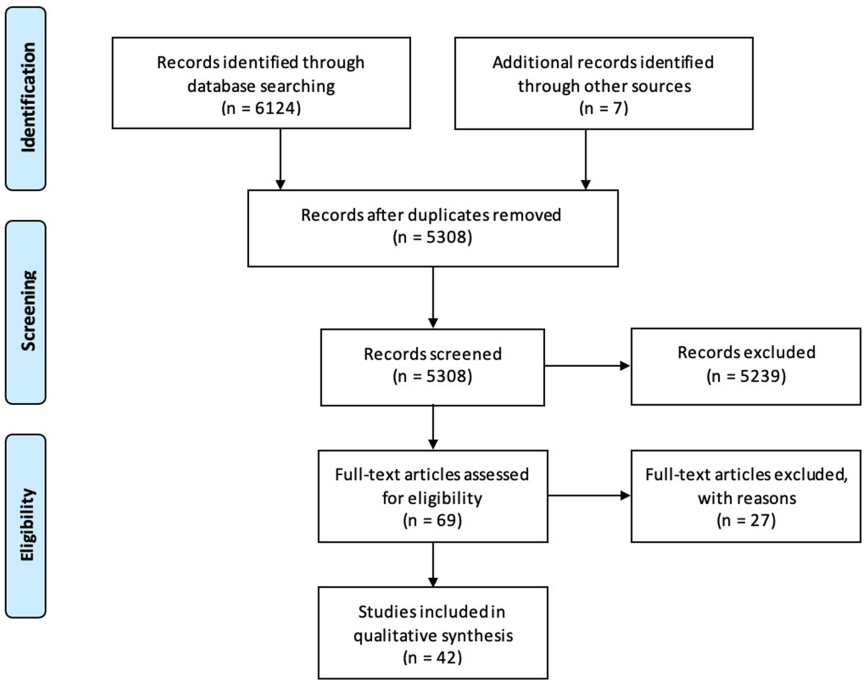

FIGURE 1 | PRISMA flow chart for article inclusion.

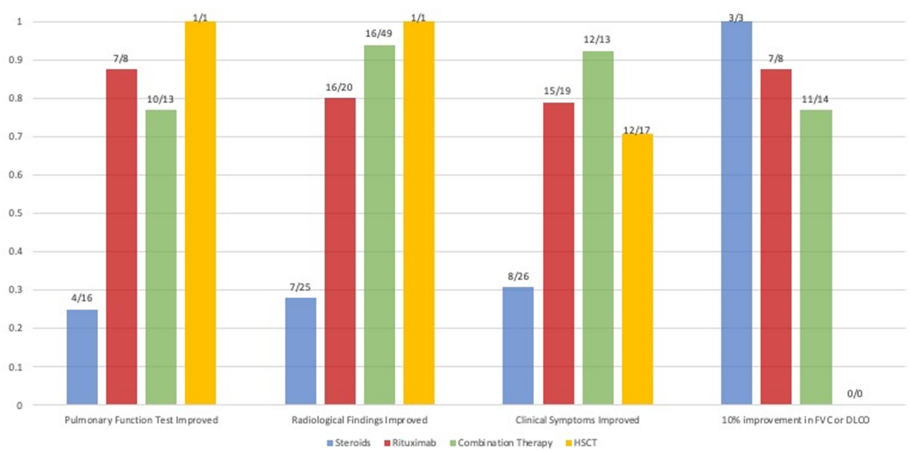

FIGURE 2 | Comparison of the available qualitative and quantitative outcomes of studies that reported on patients (N) treated with steroids, rituximab monotherapy and rituximab combination therapy. The proportion of patients that had a qualitatively reported improvement of pulmonary function tests, radiological findings and the proportion that had a quantitative improvement of their forced vital capacity (FVC) or diffusion capacity of the lung for carbon monoxide (DLCO) of 10% after therapy is shown. Due to a lack of quantitative data, statistics could not be performed.

TABLE 1 | Studies reporting treatment of GLILD in PID with corticosteroids.

Article	Study design	Sample	Intervention	Control	Qualitative outcome	Quantitative outcome
Boujaoude et al. (16)	Case study	32-year-old woman with CVID and GLILD	Prednisone at a dose of 60 mg daily, duration not mentioned	None	Improvement of CS, PFT and RF	FVC: 0.61 L increase ((% predicted increased by 19%), FEV1: 0.48 L increase
Guerrini et al. (17)	Case study	20-year-old woman with CVID and GLILD	Corticosteroids, exact duration not mentioned	None	Improvement of CS and RF	Not mentioned
Kohler et al. (18)	Case study	35-year-old woman with CVID and GLILD	Prednisone at a dose of 60 mg daily for six weeks, after which tapering was initiated	None	Improvement of PFT and RF, relapse when tapering was attempted	FVC: 0.98 L increase (% predicted increased by 28%), FEV1: 0.7 L increase
Kanathur et al. (19)	Case study	67-year-old man with CVID and GLILD	Splenectomy and prednisone at a dose of 60 mg daily for 18 months	None	No effect of prednisone at first, after splenectomy prednisone was continued, resulting in improvement of CS and RF	Not mentioned
Kaufman et al. (20)	Case study	26-year-old woman with CVID and GLILD	Prednisone at a dose of 60 mg daily for a few months, exact duration not mentioned	None	Improvement of PFT and RF	FVC: 0.08 L increase (% predicted increased by 2%) FEV1: 0.01 L increase (no change in % predicted)), DLCO: 2.9 ml/mm/mmHg (% of predicted increased by 13%)
Wislez et al. (21)	Case study	68-year old woman with CVID and GLILD	Prednisone at a dose of 0.75 mg per kg daily, then tapering to 5 mg daily over the course of two months and stopping completely eight months later.	None	Improvement of CS and RF, but relapse upon interruption of glucocorticoids. Improvement of symptoms upon reintroduction of glucocorticoids.	Not mentioned

CVID, common variable immunodeficiency; CS, clinical symptoms; DLCO, diffusing capacity; FVC, forced vital capacity; FEV1, forced expiratory volume in 1 second; GLILD, granulomatous-lymphocytic interstitial disease; MMF, mycophenolate mofetil; MTX, methotrexate; PFT, pulmonary function tests; RAG, recombination-activating gene; RF, radiological findings.

were associated with the resolution of symptoms when paired with splenectomy (19).

Conventional Disease Modifying Anti Rheumatic Drugs (DMARDs)

Besides glucocorticoids, other immunosuppressants for the treatment of GLILD have been evaluated (**Table 2**). Examples encountered in the literature included methotrexate (MTX), cyclophosphamide, mycophenolate (MMF), azathioprine, cyclosporin, hydroxychloroquine, tacrolimus and sirolimus.

Boursiquot et al. assessed the efficacy of both MTX and cyclophosphamide in the treatment of GLILD. The researchers prospectively followed 59 patients with CVID, of whom 30 had GLILD. Different treatment regimens were initiated in 25 patients with CVID and GLILD (**Table 2**). Complete remission was obtained in three (out of 13) patients who were treated with glucocorticoids, one (out of one) who was treated with MTX and one (out of five) who was treated with cyclophosphamide. Ten patients had a partial response and the remainder showed no effect at all (23).

Other articles reported the use of MMF for the treatment of GLILD. Bucciol et al. described three patients with GLILD. Glucocorticoids were ineffective, but a switch to MMF resulted in stabilization of symptoms and improvement of clinical and radiologic findings in all three cases (25). More evidence was provided by Tashtoush et al., who published a case report about a 51-year old woman with CVID and GLILD. This patient achieved remission after induction therapy with glucocorticoids for 3 months and MMF maintenance therapy for 9 months (30).

As emerged from the Delphi Study of the British Lung Foundation/United Kingdom Primary Immunodeficiency Network, azathioprine is another drug that is often used for the treatment of GLILD. An article dating back to 1996 by Sacco et al. reported the case of a six-year-old girl with CVID and severe GLILD. The patient was treated with glucocorticoids with good effect, but tapering of the medication resulted in disease relapse. This prompted the physicians to add azathioprine, which halted disease progression. The combination of prednisone and azathioprine was maintained for three years, after which they

TABLE 2 | Studies reporting treatment of GLILD in antibody deficiencies with various immunosuppressants.

Article	Study design	Sample	Intervention	Control	Qualitative outcome	Quantitative outcome
Ardenitz et al. (22)	Prospective follow up cohort study	37 patients with CVID and granulomatous disease, of which 20 also had GLILD	Splenectomy was performed in nine patients, 29 patients were given glucocorticoids, with or without other therapies, 10 subjects were also given one or more additional immune suppressants: hydroxychloroquine (five subjects), cyclosporine (three subjects), azathioprine (two subjects), methotrexate (two subjects), infliximab (one subject), and etanercept (one subject). One patients was administered rituximab. Five patients received no treatment. Duration of treatments varied. Treatment of 13 patients with GLILD was specifically reported. Patient 04: prednisone and hydroxychloroquine Patient 08: cyclosporine at a dose pf 100 mg twice daily, years of prednisone, IV glucocorticoids Patients 11: monthly oral and IV glucocorticoids Patient 14: chronic prednisone at a dose of 20 mg daily Patient 20: oral prednisone for 12 months Patients 21 oral prednisone for 12 months Patient 24: infliximab, hydroxychloroquine at a dose of 200 mg twice daily for 15 years Patient 28: MTX at a dose of 7.5 mg weekly for 12 months, hydroxychloroquine at a dose of 200 mg twice daily for five years Patient 34: years of prednisone, hydroxycholoroquine Patient 35: years of steroids at a dose of 10 mg every two days Patient 36: oral steroids at a dose of 5 mg daily for one week, COX2 inhibitors	Patients with same disease received different treatments	Outcomes were not reported for single patients. 10 (28.5%) patients died (seven of pulmonary complications and at least five with GLILD), rituximab led to resolution of autoimmunity, unclear how other drugs were effective	Not mentioned
Boursiquot et al. (23)	Prospective follow up cohort study	59 patients with CVID of which 30 also had GLILD	25 treatment regimens were noted. Oral corticosteroids were administered to 13 patients for a median of 18 months, six received cyclophosphamide for a median of six months, hydroxychloroquine was used in four cases for a median of 13.5 months, rituximab in three for a median of six months. MTX for a median of 38 months, thalidomide for a median of two months, infliximab and azathioprine were each used in two patients for a median of 31 and 18 months respectively. Cyclosporine, Interferon alpha, MMF and sirolimus were used in one patient each, for a median of 12, six, 20 and 12 months	31 patients with CVID who did not receive any treatment	Complete remission was obtained in three patients who were treated with corticosteroids, one who was treated with MTX and one who was treated with cyclophosphamide. 10 patients had a partial response and 10 had no effect at all	Not mentioned
Bouvry et al. (24)	Prospective follow up cohort study	20 patients with CVID and GLILD	17 patients received IVIg, 15 corticosteroids, three others not specified immunosuppressants and two hydroxychloroquine, duration not specified	60 patients with sarcoidosis	Six of the patients with CVID and GLILD died, all of the patients with sarcoidosis were still alive	Not mentioned
Bucciol et al. (25)	Case study	Three patients with CVID and GLILD: 23-year-old man, 18-year-old man and 4-year-old girl	Corticosteroids, duration not specified MMF, duration not specified	None	Resistance to steroids or relapse despite steroids. Stabilization of CS and improvement of RF after MMF administration	Pt 1; FVC: (% predicted decreased by 7%, FEV1: (% predicted decreased by 4%. Pt 2: Pre-treatment data not mentioned, FVC after treatment 60% of predicted FEV1 after treatment 68% of predicted Pt 3: not mentioned
Cha et al. (26)	Prospective follow-up cohort study	15 patients with various underlaying diseases (one	Corticosteroids, MTX, colchicine, azathioprine, cyclophosphamide and cyclosporin. Patient with GLILD: corticosteroids and MTX, later switched to cyclosporin, duration not mentioned	None	Patient with CVID: still alive, no effect of corticosteroids and MTX, improvement of CS and PFT when switched to cyclosporin	Not mentioned

(Continued)

TABLE 2 | Continued

Article	Study design	Sample	Intervention	Control	Qualitative outcome	Quantitative outcome
		had CVID)and GLILD)				
Davies et al. (27)	Case study	34-year-old woman CVID and GLILD	Prednisone at a dose of 40 mg daily Cyclosporin at a dose of 125 mg daily	None	No effect of prednisone, improvement of CS and RF on cyclosporin A	FVC: 0.71 L increase ((% predicted increased by 30%), FEV1: 0.6 L increase
Deya-Martinez	Case study	2 patients (12-year-old boy with CVID and GLILD and 16-year-old girl with Kabuki syndrome and GLILD)	Pt 1: rituximab at a dose of 375 mg per m2 weekly for 4 weeks twice. MMF and sirolimus at dose of 2.5 mg/m2 daily, duration not specified Pt 2: sirolimus, duration not specified	None	Pt 1: Good effect of rituximab initially, but relapse six months after treatment. Improvement of with MMF and sirolimus. Pt 2: Improvement of RF with sirolimus	Not mentioned.
Franxman et al. (28)	Case series	3 patients with CVID and GLILD (14-year-old female, 55-year-old female and a 16-year-old male)	Pt 1: Corticosteroids and MMF, dose and duration not specified. Infliximab 5 mg/kg every 4 weeks for 4 months Pt 2: Corticosteroids and plaquenil, dose and duration not specified. Infliximab 5 mg/kg every 4 weeks for 6 months Pt 3: Corticosteroids, dose and duration not specified. Infliximab 5 mg/kg every 4 weeks for 5 months		Pt 1: No effect of corticosteroids, after initiation of infliximab steroids could be tapered and there was improvement of CS, PFT and RF. Pt 2: Decline of RF PFT and CS during corticosteroid therapy. Improvement of CS & PFT. Discontinuation of treatment due to possibly treatment related skin lesions. Pt 3: Relapse upon tapering of steroids. Improvement of CS & PFT and successful taper of steroids after infliximab introduction	Pt 1; FVC: increased by 22%, FEV1: increased by 20% Pt 2; FVC: increased by 6%, DLCO: increased by 33%. Pt 3; FVC: increased by 35%
Sacco et al. (29)	Case study	Six-year-old girl with CVID and GLILD	Corticosteroids at a dose of 2 mg per kg daily for two weeks, after which tapering was started. A dose of 0.75 mg per kg daily was maintained for three years, until it was further tapered to 0.17 mg per kg per day. Azathioprine at a dose of 1.5 mg daily, for the duration of three years, after which the dose was tapered to 0.75 mg per kg per day	None	Improvement of clinical symptoms and RF with corticosteroids only, but relapse when tapering. Addition of azathioprine stabilised situation	Not mentioned
Tashtoush et al. (30)	Case study	51-year-old patient with CVID and GLILD	Prednisone at a dose of 0.5 mg per kg daily for 3 months MMF at a dose of 1000 mg daily for nine months	None	Improvement of CS and RF after 3 months	Not mentioned
Thatayatikom et al. (31)	Case study	22-year-old man with CVID and GLILD	High-dose methylprednisolone Infliximab at a dose of 10 mg daily for six weeks. After relapse treatment with infliximab was re-initiated at a dose of 5 mg daily for nine months	None	No effect of methylprednisolone, improvement after addition of infliximab, then relapse with interruption of treatment. Again, improvement of CS and RF after therapy re-initiation	Not mentioned

CVID, common variable immunodeficiency; CS, clinical symptoms; DLCO, diffusing capacity; FVC, forced vital capacity, FEV1, forced expiratory volume in 1 second; GLILD, granulomatous-lymphocytic interstitial disease; MMF, mycophenolate mofetil; MTX, methotrexate; PFT, pulmonary function tests; RAG, recombination-activating gene; RF, radiological findings.

TABLE 3 | Studies reporting treatment of GLILD in PID with rituximab.

Article	Study design	Sample	Intervention	Control	Qualitative outcome	Quantitative outcome
Arraya et al. (32)	Case report	57-year-old female with CVID and GLILD	Rituximab at a dose of 375 mg/m2 weekly for four cycles. Three cycles were used for induction, a yearly cycle was used for maintenance for 8 years.	None	Improvement of RF	Not mentioned
Ceserer et al. (33)	Case series	Three patients with CVID and GLILD (38- and 56-year-old women, 44-year-old man)	Rituximab at a dose of 375 mg/m2 weekly for four cycles. At total of 16 infusions was given	None	Improvement of CS, PFT and RF	Pt 1; FVC: 0.37 L increase ((% predicted increased by 11%), DLCO: 0.6 ml/mm/mmHg increase ((% predicted increased by 8%), FEV1: 3.04 L increase ((% predicted increased by 38%) Pt 2; FVC: 0.36 L increase ((% predicted increased by 24%), DLCO: 0.4 ml/mm/mmHg increase ((% predicted increased by 7%), FEV1: 0.19 L increase ((% predicted increased by 12%) Pt 3: FVC: 0.25 L decrease ((% predicted decreased by 4%), DLCO: 0.9 ml/mm/mmHg increase ((% predicted increased by 9%), FEV1: 0.36 L decrease ((% predicted decreased by 7%).
Maglione et al. (10)	Prospective cohort study	11 patients with CVID and progressive GLILD	Rituximab at a dose of 375 mg/m2 weekly for four cycles	44 patients with CVID but no GLILD, 14 patients with CVID and stable GLILD and four patients with CVID and progressive GLILD	Improvement of CS and RF. Relapse of 4 patients.	Not mentioned
Ng et al. (34)	Case study	Two patients with CVID and GLILD (36-year-old man and 33-year-old woman)	Corticosteroids, duration not specified Rituximab at a dose of 375 mg/m2 weekly for four cycles with a four- to six-month interval. A total of 16 infusions was given	None	Corticosteroids led to short-lived improvement of CS, rituximab led to improvement of CS and RF	Not mentioned
Tessarin et al. (35)	Case study	37-year-old woman with CVID and GLILD	Rituximab at a dose of 375 mg/m2 every four weeks, weekly for four cycles with a four to six month interval	None	Improvement of CS and RF	Not mentioned
Vitale et al. (36)	Case study	37-year-old woman with CVID and GLILD	High-dose corticosteroids, duration not specified Rituximab at a dose of 375 mg/m2 every four weeks, weekly for four cycles with a four to six month interval	None	Corticosteroids had no direct effect, addition of rituximab led to improvement of CS, PFT and RF	Not mentioned
Zdziarsky and Gamian (37)	Case study	25-year-old woman with CVID and GLILD	Methylprednisone at a dose of up to 50 mg daily, duration not specified Rituximab at a dose of 150 mg/m2 weekly for six cycles and later at a dose of 375 mg/m2 every 21 days for four cycles with a six-month remission interval	None	No effect of corticosteroids, improvement after first underdosed cycle of rituximab followed by relapse, improvement of CS and RF after second cycle of rituximab	FVC: 1.21 L increase

CVID, common variable immunodeficiency; CS, clinical symptoms; DLCO, diffusing capacity; FVC, forced vital capacity, FEV1, forced expiratory volume in 1 second; GLILD, granulomatous-lymphocytic interstitial disease; PFT, pulmonary function tests; RF, radiological findings.

were tapered to 5 mg every other day and 0.75 mg per kg daily, respectively (29).

Albeit less frequently reported, several articles describe the use of cyclosporine for the treatment of GLILD. Davies et al. reported the case of a 34-year old woman with CVID and GLILD who responded well to glucocorticoid therapy, but had recurrent relapses after tapering. The patient was eventually treated with cyclosporine, with good effect (27). Similar results were

observed by Cha et al.: a patient with CVID and concomitant GLILD was initially treated with glucocorticoids, but achieved disease remission only when therapy was switched to cyclosporin (26).

Deya-Martinez et al. showed that the immunosuppressant sirolimus can be useful in the treatment of GLILD. A boy with CVID and GLILD, who had been previously treated with rituximab and who had relapsed, was switched to sirolimus monotherapy and achieved remission of symptoms (39).

Two articles reported the use of DMARDs for the treatment of GLILD in relatively large patient series. Both papers described variable regimens of multiple drugs, without mentioning the outcomes.

Ardeniz described the long-term follow up of a group of 37 patients with CVID and granulomatous disease, of which 20 patients had GLILD. Patients were treated with a different combination of drugs, including glucocorticoids, cyclosporine, hydroxychloroquine, infliximab, etanercept and rituximab. Outcomes were not clearly reported. Over the follow-up period of 25 years, 10 of the 37 patients included in the study died. Of those, at least five had GLILD (22).

Bouvry compared outcomes of CVID patients with GLILD with those of patients with sarcoidosis. Patients were treated with different immunosuppressants over the course of the study. Results were not clearly reported, the main difference between the two groups was that patients with CVID and GLILD had worse outcomes than those with sarcoidosis (24).

Biologicals

Biologicals, also known as biological medicinal products, are drugs which are (partially) produced by living organisms by means of recombinant DNA technologies (40). For GLILD specifically, infliximab, rituximab and abatacept have been used.

Infliximab

Infliximab is a monoclonal antibody that binds to TNFα and blocks signaling, thus interfering with a central mechanism of inflammation (41). Thatayatikom et al. reported a 22-year-old man with CVID and life-threatening GLILD, who was first unsuccessfully treated with glucocorticoids, but achieved remission after treatment with infliximab for nine months (31). Additionally, Franxman, Howe & Baker described three patients who all showed remission of GLILD on CT scan and pulmonary function tests, after 4 months, 8 months and 5 months of treatment, respectively (28).

Rituximab

Rituximab is a monoclonal antibody that depletes B-cells, by binding to CD20 molecules on their surface (42). Seven studies focused on rituximab monotherapy for GLILD (**Table 3**). Arraya, Cereser, Ng and Tessarin all reported cases of patients with CVID and GLILD who were successfully treated with rituximab monotherapy (at a dose of 375 mg/m2 weekly for four weeks) (32–35). Maglione et al. followed 73 patients for 18 months: 44 patients had CVID only, 14 had concomitant stable GLILD, and 15 had concomitant progressive GLILD. 11 of the 15 patients with progressive GLILD were treated with rituximab at a dose of 375 mg/m2 weekly for four weeks: all experienced stabilization or improvement of disease activity, however four relapsed 18 months after completion of therapy (10).

Of particular interest is the study by Zdziarsky and Gamian's, describing a 25-year old woman with CVID and GLILD who was treated with rituximab monotherapy at a relatively low dose of 150 mg/m2 weekly for six weeks because of risk of infection (37). This resulted in incomplete remission of clinical symptoms, and the patient relapsed six months later. Treatment with rituximab was repeated, this time at a dose of 375 mg/m2, resulting in complete remission for a period of 30 months.

Combination Chemotherapy With Rituximab and Azathioprine

Eight studies evaluated combination chemotherapy with rituximab and azathioprine (**Table 4**). The rationale behind this combination chemotherapy is that B- and T-lymphocytes are targeted simultaneously (38). Chase and colleagues were the first ones to pioneer this approach. They performed a longitudinal prospective cohort study in which they followed seven patients with CVID and GLILD, who were treated with intravenous rituximab and oral azathioprine for 18 months. All patients experienced some degree of improvement in radiological findings (38). These results were confirmed by Pathria, Routes, Limsuwat and Tillman, who reported successful treatment of patients with CVID and GLILD with combination chemotherapy (44–46, 49). Vitale et al., reported successful addition of combination therapy with rituximab to glucocorticoid treatment in a 17-year old patient with CVID and GLILD after initial unresponsiveness to glucocorticoid monotherapy (36). Jolles' and Sood's articles showed that azathioprine can be replaced by other drugs with similar mechanisms of action. For example, Jolles et al. described a 51-year old woman with CVID and GLILD treated with a combination of rituximab and MMF, because of intolerance of azathioprine. Five months into treatment, the patient experienced an improvement of symptoms, alongside better pulmonary function and radiologic results (43). Sood et al. reported an improvement of GLILD related symptoms in the case of a 16-year old boy with 22q.11 deletion syndrome who was treated with rituximab and 6-mercaptopurine (48). One additional article by Verbsky et al. was added to the review despite its publishing date (June 2020) being after the last literature search (March 2020). We choose to mention this article, because the planned publication of the paper was known to the authors at the time of the literature search and, most importantly, because its results are highly relevant for this systematic review. The authors performed a retrospective chart review of 39 patients with CVID and GLILD who were treated with a combination of rituximab and azathioprine or rituximab and MMF. The median follow-up period was four years. 37 patients were included in the final analysis and of those 34 (92%) experienced an improvement of GLILD-related parameters. 27 patients (73%) experienced sustained remission, whereas nine patients (24%) relapsed after a median of 3.2 months. Of those relapsing, two patients died of septicemia and respiratory failure, respectively (47).

TABLE 4 | Studies reporting treatment of GLILD in antibody deficiencies with combination chemotherapy.

Article	Study design	Sample	Intervention	Control	Qualitative outcome	Quantitative outcome
Chase et al. (38)	Prospective follow-up cohort study	Seven patients with CVID and GLILD	Five patients received corticosteroids Rituximab at a dose of 375 mg/m2 weekly for four cycles with a four to six month interval. A total of 12-16 infusions was given Azathioprine at a dose of 1-2 mg per kg for 18 months	None	No effect of corticosteroids, combination chemotherapy led to improvement of CS and RF	Pt 1; FVC: 0.52 L increase ((% predicted increased by 9%), FEV1: 0.3 L increase ((% predicted increased by 9%), DLCO 6.89 increase ((% predicted increased by 27%).Pt 2; FVC: 0.4 L increase ((% predicted increased by 13%), FEV1: 0.11 L increase ((% predicted increased by 6%), DLCO after treatment 22.1 (98% of predicted).Pt 3; FVC: 0.11 L increase ((% predicted increased by 2%), FEV1: 0.09 L increase ((% predicted increased by 2%), DLCO 5.3 decrease ((% predicted decreased by 19%).Pt 4; FVC: 0.4 L increase ((% predicted increased by 5%), FEV1 0.4 L increase ((% predicted increased by 7%), DLCO 2.9 increase ((% predicted increased by 9%).Pt 5; FVC: 0.22 L decrease ((% predicted decreased by 4%), FEV1: 0.14 L decrease ((% predicted decreased by 2%), DLCO: 0.51 increase ((% predicted increased by 3%).Pt 6; FVC: 1.22 L increase ((% predicted increased by 33%), FEV1: 0.97 L increase ((% predicted increased by 31%), DLCO after treatment 19.00 (76% of predicted).Pt 7; FVC: 0.73 L (18% of predicted), FEV1 0.49 L (16% of predicted), DLCO 6.6 increase (20% of predicted).
Jolles et al. (43)	Case study	51-year-old woman with CVID and GLILD	Rituximab in two doses of 1g MMF for seven months	None	Improvement of PFT and RF	FVC: % predicted increased by12.5%, DLCO: % predicted increased by 10.9%
Limsuwat et al. (44)	Case study	56-year-old man with CVID and GLILD	Rituximab at a dose of 375 mg/m2 for four weeks, followed by azathioprine 200 mg/d	None	Improvement of CS, CT and PFT	FVC: 1.0 L increase (53% increase), FEV1: 0.45 L increase (46% increase)
Pathria et al. (45)	Case study	61-year old woman with CVID and GLILD	Rituximab at a dose of 375 mg/m2 was initiated. A total of four infusions were given Azathioprine at a dose of 0.75 per kg, which was increased to 1.5 mg per kg after two months	None	Improvement of CS and RF	Not mentioned
Routes and Verbsky (46)	Case study	17-year old girl with CVID and GLILD	Corticosteroids for other auto-immune manifestations Rituximab and azathioprine (dose not mentioned)	None	Improvement of PFT & RF	Not mentioned
Verbsky et al. (47)	Retrospective cohort study	37 patients with CVID and GLILD	One patient received glucocorticoids prior to combination chemotherapy (dose not mentioned) Rituximab at a dose of 375 mg/m2 weekly for four cycles with a four to		Glucocorticoids had no effect. Improvement of RF in 34/37 (92%) after combination chemotherapy. Remission was maintained in 27 patients, 9 had relapses after a median of 3.2 years, one patient underwent lung	At baseline, FEV1 and FVC were normal in 16 (41%) patients, restrictive in 17 (44%), obstructive in 2 (%%) and mixed obstructive-restrictive in 4 (10%). 29 GLILD had DLCO measurements, 14 were normal (48%)*

(Continued)

TABLE 4 | Continued

Article	Study design	Sample	Intervention	Control	Qualitative outcome	Quantitative outcome
			six-month interval. A total of 16 infusions was given Azathioprine at a dose of 1-2 mg per kg daily or MMF at a dose of 250-1000 mg twice daily for a median of 16 months		transplantation. Two patients eventually died, one of septicemia seven months after completion of treatment and the other of respiratory failure (not mentioned at which timepoint after treatment)	
Sood et al. (48)	Case study	16-year old boy with 22q.11 deletion syndrome, CVID and GLILD	Corticosteroids for other auto-immune manifestations Rituximab at a dose of 375 mg/m2 6-Mercaptopurine at a dose of 0.5 mg per kg three times weekly	None	Improvement of CS	Not mentioned
Tillman et al. (49)	Case study	13-year-old girl with CVID and GLILD	Rituximab at a dose of 375 mg/m2 weekly for four cycles Azathioprine at a dose of 50 mg once daily for 18 months	None	Improvement of CS and RF	FVC: increase of 64% of predicted FEV1: increase of 49% of predicted
Vitale et al. (36)	Case study	17-year-old boy with CVID and GLILD and intracranial lymphoproliferative lesions	High-dose corticosteroids Rituximab at a dose of 375 mg/m2 weekly for four cycles with a four to six-month interval. A total of 16 infusions was given Azathioprine at a dose of 1.7 mg per kg for 18 months	None	Corticosteroids had no effect, rituximab led to improvement of CS and RF with resolution of intracranial lesions	FVC: 0.62 L increase, FEV1: 0.54 L decrease

**In the paper by Verbsky et al. (47), the total number of patients included are 39, the total number of patients treated with combination chemotherapy were 27.*
CVID, common variable immunodeficiency; CS, clinical symptoms; DLCO, diffusing capacity; FVC, forced vital capacity; FEV1, forced expiratory volume in 1 second; GLILD, granulomatous-lymphocytic interstitial disease; MMF, mycophenolate mofetil; PFT, pulmonary function tests; RF, radiological findings.

Abatacept

CTLA-4 haploinsufficiency and LRBA deficiency result in a phenotype similar to CVID with severe immunodeficiency, lymphoproliferation and autoimmunity. In the physiological state, T lymphocyte responses are regulated by binding of the B7 ligand to CTLA-4 thus blocking T-cell activation, whereas LRBA is involved in intracellular trafficking and, among others, preserves CTLA-4 from degradation (50, 51), causing excessive immune activation. Abatacept consists of the Fc region of immunoglobulin IgG1 fused to CTLA-4 (52) and thus serves as a CTLA-4 fusion protein preventing excessive T lymphocyte proliferation in patients with CTLA-4 haploinsufficiency and LRBA deficiency.

A total of three articles described the use of abatacept for the treatment of GLILD (**Table 5**). Schwab and colleagues performed a longitudinal prospective cohort study in which they followed 133 patients with CTLA-4 haploinsufficiency. Of these, two patients who presented with GLILD treated with abatacept

TABLE 5 | Studies reporting treatment of GLILD in PID with abatacept.

Article	Study design	Sample	Intervention	Control	Qualitative outcome	Quantitative outcome
Kostel Bal et al. (53)	Case study	7 patients with LBRA deficiency, one of which had concomitant GLILD (12-year-old boy)	Abatacept at a dose of 20 mg per kg every two weeks, duration not specified	None	Improvement of RF	Not mentioned
Lo et al. (54)	Prospective follow-up cohort study	Nine patients with LBRA deficiency, three of whom also had GLILD	Corticosteroids and MMF, duration not specified Abatacept in different doses: 20 mg per kg every two weeks, 20 mg per kg every four weeks, 30 mg per kg monthly for six months	None	Disease progression despite treatment with corticosteroids and MMF Improvement in clinical symptoms, PFT and RF	Pt 1: FVC: % predicted increased by 30-40%, FEV1: % predicted increased 35%, DLCO % predicted increased by 35%. Pt 3: FVC: % predicted increased by 50% of predicted, FV1: % predicted increased by 40%, DLCO % predicted increased by 50%.
Schwab et al. (51)	Prospective follow-up cohort study	90 CTLA4 mutation carriers, of which 32 with GLILD	Abatacept was administered to 14 patients, duration not specified	43 unaffected mutation carriers	Six of the patients treated with abatacept experienced improvement of symptoms (two who had GLILD had resolution of lymphoproliferative lesions)	Not mentioned

experienced improvement of both clinical symptoms and radiologic findings (51).

Lo and colleagues reported three patients with LRBA deficiency and GLILD, who experienced significant improvements in lung function and radiological findings after treatment with abatacept (54). Bal replicated these results, findings abatacept to be useful in the treatment of GLILD in a 12-year old boy with LRBA deficiency (53).

Hematopoietic Stem Cell Transplantation

HSCT holds the promise of being a definitive treatment for GLILD as it can correct the underlying immunodeficiency and the associated GLILD instead of just alleviating GLILD related symptoms. However, it is associated with considerable risks, including Graft versus Host Disease (GvHD) and serious infections, both associated with considerable morbidity. This risk is likely higher in those with established structural lung disease.

Five studies reported on HSCT for CVID patients with associated GLILD (**Table 6**). Wehr followed 25 patients with CVID who underwent HSCT. Five patients had GLILD: four experienced an improvement of the CVID-related complications; one died 104 days after transplantation due to acute GvHD and infectious complications (60). Wehr's papers also includes four patients which were discussed in Rizzi's publication in 2011 (56). Hartono published the case of a 23-year-old woman who presented with a CVID-like phenotype due to a STAT1 gain-of-function mutation and GLILD: after HSCT there was an improvement of radiologic findings (55). Mixed outcomes were reported by both Seidel and Tesc. Seidel and colleagues performed an international survey and collected information about 12 patients with CVID-like disease due to underlying LRBA deficiency (seven of whom also had GLILD), who underwent HSCT. Four patients went into partial remission, whereas three of them died (57). Tesch published a prospective follow-up study of 76 patients with LRBA deficiency, of which 24 underwent HSCT. Of these 24 patients, 17 of the 24 patients survived and all of the seven patients with concomitant GLILD experienced an improvement of GLILD related symptoms. Two patients who did not have GLILD before HSCT, developed the disease after the procedure (59).

Quality of Studies and Level of Evidence

All studies had an overall intermediate or high risk of bias (**Table 7**). This was largely due to the small sample sizes and lack of controls. Outcomes were mostly reported qualitatively, with few data about pulmonary function tests and a lack of standardized CT evaluation. The duration of follow-up was typically limited, meaning that long-term outcomes of patients remained uncertain. As far as confounders are concerned, smoking status was not always reported. Finally, genetic testing for CTLA-4 haploinsufficiency and LRBA deficiency only became available as of 2012, meaning that older articles could not make this additional distinction.

In 27 studies the level of evidence was 4, and in 12 studies the level of evidence of 3. The associated level of practice recommendations was weak in both groups.

DISCUSSION

To our knowledge, this is the most comprehensive systematic review analyzing treatment efficacy for GLILD in CVID. We show that there is still much uncertainty about the optimal treatment for GLILD and that more basic scientific and clinical research is needed in order to establish the best standard of care.

There are many factors influencing the choice of treatment. Apart from efficacy, risk-to-benefit ratio and patient preference, drug availability and cost may also play a role. Several studies reported that the efficacy of glucocorticoid monotherapy is limited. Other immunosuppressants were often used as second-line therapy with varying results. Rituximab monotherapy and combination chemotherapy with rituximab

TABLE 6 | Studies reporting treatment of GLILD in antibody deficiencies with HSCT.

Article	Study design	Sample	Control	Donor	Conditioning*	GVHD prophylaxis	Outcome (GLILD)	Outcome (Survival)
Hartono et al. (55)	Case study	23-year old girl with STAT1 mutation and GLILD	None	MUD	Not mentioned	Steroids	Improvement of radiological findings	Patient still alive day +522 post-transplant
Rizzi et al. (56)	Case study	One patient with CVID and GLILD	None	Patient 004: MUD	Patient 004: RIC[1]	CsA	Subjective improvement of PFT and reduction of steroids use	Patient with GLILD survived
Seidel et al. (57)	Prospective follow up cohort study	12 patients with LBRA deficiency of which seven also had GLILD	None	Patient 001: MFD Patient 002: MSD Patient 004: MUD Patient 006: MMFD Patient 008: MUD Patient 010: MUD Patient 01: MSD	Patient 001 RIC[2] Patitent 002 RIC[3] Patient 004 RIC[4] Patitent 006 RIC[5] Patient 008 RIC[6] Patient 010 RIC[7] Patient 011 RIC[8]	Not mentioned	Patients 002 and 010 with GLILD had complete remission (no symptoms and no need for medication), patient 001 with GLILD had good partial remission (some symptoms but no need for medication), patient 011 with GILD had partial remission (improvement of symptoms but still need for medication)	Overall survival was 67% (8/12). Patient 004, 006 and 008 with GLILD died three and two months post procedure
Slatter et al. (58)	Prospective follow up cohort study	Two patients with CTLA4 deficiency and GLILD	None	MUD	Not mentioned	Five patients (1, 2, 5, 6, and 8) CsA and MMF for GVHD. Three (3, 4, and 7) had CsA alone, CsA and MMF, or MTX and tacrolimus. Patient 6 had prednisolone, sirolimus, and belatacept until 8 days before transplant	Improvement of symptoms, tapering of immunosuppressive medication.	Six patients are still alive (two patients with GLILD fall in this group and are alive and well at 4 months and 4 years post-transplantation), two died of GvHD and DKA, respectively
Tesch et al. (59)	Prospective follow up cohort study	76 patients with LBRA deficiency of which 24 underwent HSCT and 17 had GLILD	Patients who did not undergo HSCT	Patient 001: MMUD Patient 002: MSD Patient 003: MSD Patient 004: MSD Patient 005: MFD Patient 007: MSD Patient 010: MUD Patient	Patient 001 RIC[9] Patient 002 MAC[10] Patient 003 RIC[11] Patient 004 RIC[12] Patient 005 RIC[13] Patient 007 RIC[14] Patient 010 RIC[15] Patient 014 RIC[16]	Not mentioned	Of the eight patients with GLILD, five are in complete remission, two are in partial remission with still some symptoms of GLILD. Of the 24 patients undergoing HSCT, two developed GLILD after the procedure	Overall survival was 70.8% (17/24)

(Continued)

TABLE 6 | Continued

Article	Study design	Sample	Control	Donor	Conditioning*	GVHD prophylaxis	Outcome (GLILD)	Outcome (Survival)
Wehr et al. (60)	Prospective follow-up cohort	Two patients with CVID and GLILD	None	014: MSD Patient 004: MUD Patient 029: MUD	Patient 004: RIC[17] Patient 028: MAC[18]	Patient 004: CsA Patient 028: CsA, sirolimus, MMF, corticosteroids	Patient 004: not mentioned Patient 028: deceased	Patient 028 died 104 days after procedure of aGvHD and infectious complications

Ale: Alemtuzumab; ATG: anti-thymocyte globulin; Bu: Busulfan; CsA: Cyclosporin A; CP: cyclophosphamide;Flu: Fludarabine; MAC: myeloablative conditioning; Mel: Melphalan; MFD: matched family donor;MMFD: mismatched family donor; MMUD: mismatched unrelated donor; MSD: matched sibling donor; MUD: matched unrelated donor; RIC: reduced intensity conditioning.

Conditioning: only conditioning regimens for patients with PADs were reported. [1]Flu, Mel and Ale,[2]Flu, ATG, Treo, [3]Flu, ATG ,[4]Flu, ATG, Treo, Thiotepa,[5]Flu, ATG, Thiotepa, Mel, [6]Flu, ATG, Mel, [7]Flu, ATG, Thiotepa, [8]Flu, ATG, Treo, [9]Fly, ATG, Mel, [10]CP, Bu, [11]Flu, ATG, Mel, [12]Flu, ATG, Mel, [13]Flu, ATG, Treo, Thiotepa, [14]Flu, ATG, Treo, Thiotepa, [15]Flu, ATG, Treo, Thiotepa, [16]Flu, ATG, Mel, [17]Flu and Mel, [18]Bu and Flu,*

and azathioprine emerged as promising second-line treatments. Abatacept has been used in patients with CTLA-4 and LRBA mutations, but has not been routinely used in other patient populations as of yet. Finally, HSCT may be an option when other treatments have failed, but reported survival after HSCT in CVID has been poor.

Our findings suggest that glucocorticoids, although widely used as first line therapy, failed to induce remission in 57% (17 individuals) of patients using glucocorticoids (18, 23, 26, 27, 31, 36–38). Treatment with glucocorticoids led to a partial response in 13% (four individuals) and failed to maintain remission in 7% (two individuals) of patients (18, 29). There are, however, also literature reports about the positive effects of glucocorticoids (16, 17, 20, 21). 23% (seven individuals) of all patients using glucocorticoids had resolution of symptoms. It is currently unclear how much reporting bias has occurred in the reports describing the use of for example glucocorticoids for treatment of GLILD. Based on current knowledge, it remains unclear how the benefits of glucocorticoids in some patients may weigh against the side-effects of long-term treatment.

With respect to the category of the (biological) DMARDs, MMF, azathioprine, cyclosporine, sirolimus and infliximab have demonstrated efficacy in single case reports. Yet, because of the anecdotal nature of the studies and the relatively small patient populations they were described in, there is insufficient evidence to make definitive statements. While a previous survey has shown that most physicians agree on the implementation of azathioprine and MMF, there is no consensus as far as other (biological) DMARDs are concerned (9).

We found that rituximab monotherapy was effective in treating GLILD in most cases, although relapses did occur after B cell reconstitution (10, 39). Combination chemotherapy with rituximab and azathioprine is another potential treatment regimen in patients with CVID and GLILD. Our collected data show that this combination of drugs was effective at inducing remission in all cases, even where other therapies had failed (36–38). However, there are also indications that upon prolonged follow-up, relapses may occur (10, 47). The findings on rituximab are in line with published literature which indicates both rituximab and rituximab-based chemotherapy are effective treatments for GLILD in CVID (9). The current literature does not allow to determine whether rituximab monotherapy is superior, equally effective or inferior to rituximab-based combination chemotherapy.

Abatacept is often implemented in the treatment of GLILD in patients with CTLA-4 haploinsufficiency and LRBA deficiency. Results were promising as the drug was effective in most reported cases. Although abatacept is mostly implemented for the treatment of patients with CTLA-4 or LRBA related diseases, it would be interesting to see whether it could be of benefit in other GLILD patient populations as well.

HSCT is a potentially curative treatment for immunodeficiencies and GLILD, yet is associated with the risk of serious complications. Our results show that when successfully carried out, HSCT does indeed lead to resolution of GLILD symptoms in most cases. One exception was two patients in the study by Tesch et al., who developed GLILD after HSCT (59). On the other hand, the reported mortality rate was still relatively high compared to overall survival of patients transplanted for other types of PID. While for patients with CVID and GLILD the survival after HSCT varied between 48% and 70%, in PIDs in general it approaches 90% (61). Furthermore, the procedure of HSCT encompasses immunosuppression as a result of the conditioning and replacement of hematopoietic stem cells, and it is as yet not fully proven which of these two components is responsible for the reduction of GLILD activity after HSCT. There are many factors influencing transplantation outcome, including HLA matching, severity of pre-existing lung disease, infections and the presence of active inflammation in other organs which can make transplant more hazardous. Bone-marrow microenvironment, that is, the complex interplay of local and systemic factors driving and influencing stem cell development, has recently emerged as a potential contributor to the success or failure of HSCT. As pointed out by Troilo and colleagues, approximately half of patients with CVID undergoing HSCT experience incomplete B-cell reconstitution. By studying development and maturation of B-cells of immunodeficient patients with different genetic mutations *in vitro*, the researchers found that patients with a non-supportive bone-marrow niche may not allow for adequate immune cell reconstitution and may have worse outcomes (62). These findings

may help in in the prediction of which CVID patients with GLILD could benefit from HSCT.

Furthermore, our study did not find clear differences in treatment responses between children (27 individuals) and adults (228) with GLILD. While mortality is higher in patients with pediatric-onset disease (63) almost all literature reports of children with GLILD showed a positive response to treatment. However, in order to make a clear statement about the prognosis of pediatric-onset GLILD, long-term follow-up data would be required.

TABLE 7 | Quality of studies analyzing treatment for GLILD in primary antibody deficiencies.

	Quality of the study					Confounders				
Article	**Study Design**	**Controls**	**Outcome**	**Follow-up**	**Dose**	**Smoking**	**Age**	**Co-morbidities**	**Genetic testing**	**Overall risk of bias**
Arraya et al.	–	–	+/-	+	+	–	+	+	–	High
Ardenitz et al.	+	+	–	+	–	–	+	–	–	High
Boujaoude et al.	–	–	+	–	+	+	+	+	–	High
Boursiquot et al.	+	+	+/-	+	+/-	–	+/-	+/-	–	High
Bouvry et al.	+	+/-	–	–	–	–	+	–	–	High
Bucciol et al.	–	–	+/-	+	–	–	+	+	–	High
Ceserer et al.	–	–	+/-	+	+	–	+	–	–	High
Cha et al.	+	+/-	+/-	+	–	+	+	+	–	Intermediate
Chase et al.	+/-	–	+	+/-	+	–	+	–	+	High
Davies et al.	–	–	+	+	+	+ (non smoker)	+	+	–	Intermediate
Deya-Martinez et al.	–	–	+/-	+/-	+	-(children)	+	+	+	High
Franxman et al.	+/-	+/-	+	–	+	–	+	+	–	High
Guerrini et al.	–	–	+/-	–	–	–	+	+	–	High
Hartono et al.	–	–	+/-	+	NA	–	+	+	+	Intermediate
Jolles et al.	–	–	+/-	+	+	–	+	+	–	High
Kanathur et al.	–	–	+/-	+	+	+	+	+	–	Intermediate
Kaufman et al.	–	–	+	+/-	+	–	+	+	–	High
Kohler et al.	–	–	+	+	+	–	+	+	–	High
Kostel Bal et al.	–	–	+/-	–	+	–	+	+	+	High
Limsuwat et al.	–	–	+	+/-	+	+	+	+	–	Intermediate
Lo et al.	+/-	+/-	+/-	+	+	–	+	+	+	Intermediate
Maglione et al. (8)	–	+	+/-	–	+	–	+	+	–	High
Maglione et al. (10)	+	+	+/-	+	+	–	+	+	–	Intermediate
Ng et al.	–	–	+/-	+	+	–	+	+	–	High
Pathria et al.	–	–	+/-	–	+	+	+	+	–	High
Rizzi et al.	–	–	+/-	+	NA	–	+	+	–	High
Routes & Verbsky	–	–	+/-	–	–	–	+	+	–	High
Sacco et al.	–	–	+/-	+	+	–	+	+	–	High
Schwab et al.	–	+/-	+/-	–	–	–	+	+	+	High
Seidel et al.	+/-	–	+/-	+	NA	–	+	+	+	Intermediate
Slatter et al.	+/-	–	+/-	–	NA	–	+	+	+/-	High
Sood et al.	–	–	+/-	+/-	+	–	+	+	+	Intermediate
Tashtoush et al.	–	–	+/-	+/-	+	+ (non smoker)	+	+	–	High
Thatayatikom et al.	–	–	+/-	+	+	–	+	+	–	High
Tesch et al.	–	+	+/-	+	NA	–	+	+	+	Intermediate
Tessarin et al.	–	–	+/-	+/-	+	–	+	+	–	High
Tillman et al.	–	–	+	+	+	- (children)	+	+	–	Intermediate
Verbsky et al.	+/-	–	+	+	+	–	+	–	+	Intermediate
Vitale et al.	–	–	+	+	+	–	+	+	–	High
Wehr et al.	+	–	+/-	+/-	NA	–	+	+	–	High
Wislez et al.	–	–	+/-	–	+	+ (smoker)	+	+	–	High
Zdziarsky et al.	–	–	+/-	+	+	+ (non smoker)	+	–	–	High

Strengths & Limitations

This is the first review that comprehensively summarizes all peer-reviewed data about the treatment of GLILD in CVID. A systematic approach was implemented according to the internationally recognized PRISMA guidelines that aimed at identifying all existing literature on the treatment of GLILD in CVID. Two databases were searched and, in order to reduce the risk of bias, the screening process was carried out by two independent blinded researchers.

Despite efforts to minimize weaknesses, several limitations need discussion. First of all, there might be bias intrinsic to the published studies. Glucocorticoids are considered first-line treatment for GLILD (9), which could mean that their efficacy is taken for granted and successfully treated patients are under-reported.

Further, the definition of GLILD used throughout this paper may have some limitations. Even though we strictly adhered to the internationally recognized definition of GLILD used by the British Lung Foundation/United Kingdom Primary Immunodeficiency Network, we must acknowledge that GLILD is a spectrum of symptoms and manifestations and that the impact on daily life and response to treatment may differ accordingly. Hence, there is a certain degree of interindividual variation that is difficult to quantify in the absence of detailed and objective information, such as standard radiological scores and pulmonary function tests.

Moreover, we excluded several case reports describing patients with CVID and granulomatous disease, often classified as sarcoidosis, not fulfilling the current GLILD criteria. However, some of these patients may have suffered from GLILD. Indeed, there are several case reports describing patients who were misdiagnosed with sarcoidosis and who were frequently unresponsive to glucocorticoid monotherapy, similarly to the results described in this review (64–66).

Moreover, treatment regimens were strictly defined to enable comparison of the effects of different types of monotherapy. In addition, strict criteria for evaluation of remission of GLILD were formulated. Because of this, small positive effects of treatment might have been underreported in this study.

Finally, long-term effects of medication are seldom mentioned, including the risk of infection linked to the prolonged use of immunosuppressants. This could either mean that the added effect of immunosuppressants in already immunocompromised individuals is negligible or that there is some degree of reporting bias at play. Similarly, little to no side-effects were mentioned in the analyzed literature. However, glucocorticoids are unsuitable long-term therapy candidates because of detrimental effects on metabolism, bone density, growth and behavior. As mentioned previously, the quality of the evidence was relatively low, because none of the included studies had an experimental set-up. The choice of outcome measures was heterogeneous, and often only qualitative assessments were made, thus preventing meta-analysis. Possible confounders were rarely mentioned in the reviewed literature. Hence, it was difficult to make any final recommendations for clinical practice based on the available literature.

Future Directions

Understanding the cause of GLILD is critical in finding a cure for this disease. About 10-20% of patients with CVID develop GLILD, which suggests that the complication is brought on by a combination of (epi-) genetic and/or environmental factors rather than a single cause (7). It could be postulated that individuals with GLILD are a specific subset of the patient population with CVID, with a susceptibility for lymphoproliferation. Reverse thinking by translating from the bench back to hypothesis formulation can help assemble a workable theoretical framework. If, as is currently thought, GLILD is a form of immune dysregulation, there are potentially two important players, namely T-cells and B-cells (67).

The efficacy of second-line immunosuppressants that selectively target T-cells suggest they have an important role in the pathogenesis of GLILD. On the other hand, the successful use of rituximab in the treatment of the disease supports the idea that B-cells may be important effector cells, either initiating or maintaining inflammation in GLILD. A combined role of T- and B-lymphocytes has also been suggested: superior efficacy of the combination of azathioprine and rituximab compared to rituximab monotherapy would plead in favor of this hypothesis (38).

However, fundamental research into the pathophysiology of GLILD is needed to corroborate any of the above-mentioned hypotheses. In patients in whom monogenetic defects are identified, personalized medicine with individualized treatment strategies could be devised. Histopathological analysis, where available, may support this. Abatacept in CTLA-4 haploinsufficiency and LRBA deficiency is a good example of how personalized medicine is already being implemented in clinical practice.

In order to improve patient care and treatment of GLILD, it is important to screen for the condition, and define the best standard of treatment (9). RCTs are still lacking, because, due to the low incidence of GLILD, it is difficult to recruit sufficient numbers of participants. However, a combined effort by international consortium of medical centers, could allow for standardized data collection on a much larger scale, including pulmonary function tests and a uniform radiographic high-resolution CT scan score. Indeed, studies such as STILPAD are on-going and will inform on this. Until then, uniform standardized reporting on GLILD is crucial. Based on previous literature, this should at least include information on how the GLILD diagnosis was made, dosage and interval of the intervention, treatment-associated side effects (both short- and long-term), pre- and post-treatment CT scores using a universal scoring method, pulmonary function tests including carbon-monoxide diffusion and lymphocyte phenotyping data, ideally using validated tools. Results could provide scientific backup for current treatment strategies and help create new, evidence-based treatment protocols.

CONCLUSION

Based on this systematic review of the current literature, which was often of low quality with a high risk of bias, it is impossible to define which therapeutic option is optimal in treating GLILD in CVID.

Corticosteroid monotherapy seems suboptimal for many patients, rituximab monotherapy and combination chemotherapy with rituximab and azathioprine were effective in most reported cases. The use of abatacept has so far been only implemented as therapy for patients with pathogenic CTLA-4 and LRBA mutations. HSCT is the only curative treatment for GLILD, yet not free of risks. While much is left open and uncertain, what has become most evident throughout this review is that there remain many critical knowledge gaps concerning treatment of GLILD. Etiology and optimal treatment for the disease are questions that require urgent answers, as they may lead to better and more specific treatment regimens. In the future, larger well-designed studies evaluating therapeutic strategies should be carried out, with uniform quantitative outcomes.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

OL and BS created the search string, selected the articles included in the review, wrote the paper, and created the tables. JM chose the review topic, and guided the research and writing process. JW gave advice about the methodology and reviewed the final text. CC-R and H-eH provided additional raw data which was included in the review. VD, GB, JH, H-eH, HI, HL, ST-L, SP, AR, AS, AV, and KW gave advice during the synthesis of the results, commented on the draft papers, and reviewed the final text. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2021.606099/full#supplementary-material

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Conflict of Interest: JH and KW co-chair the European Respiratory Society-funded e-GLILDnet Clinical Research Collaboration which is a collaboration with ESID (the European Society for Immunodeficiencies).

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The reviewer EK declared a past co-authorship with one of the authors CC-R to the handling editor.

APPENDIX: SEARCH STRING

Population: patients with PID and GLILD

Intervention: treatment (pharmacological and/or stem cell transplantation)

Control: no therapy or placebo

Outcome: clinical symptoms, pulmonary function tests, radiologic findings, mortality

PubMed

"common variable immunodeficiency"[MeSH] OR CVID [Title/Abstract] OR common variable immunodeficiency [Title/Abstract] OR primary immunodeficiency [Title/Abstract] OR GLILD [Title/Abstract] OR antibody deficiency [Title/Abstract] OR granulomatous lymphocytic interstitial lung disease [Title/Abstract] OR granulomatous disease[Title/Abstract] OR interstitial lung disease [Title/Abstract] OR ILD [Title/Abstract] OR granulomatous lung disease [Title/Abstract] OR lymphocytic interstitial pneumonitis [Title/Abstract] OR lymphoid interstitial pneumonitis [Title/Abstract] OR LIP [Title/Abstract]

AND "hematopoietic stem cell transplantation"[MeSH] OR hematopoietic stem cell transplantation[Title/Abstract] OR HSCT[Title/Abstract] OR stem cell transplantation[Title/Abstract] OR SCT[Title/Abstract] OR "abatacept"[MeSH] OR abatacept[Title/Abstract] OR corticosteroid*[Title/Abstract] OR prednisone[Title/Abstract] OR methotrexate [Title/Abstract] OR "mycophenolic acid"[MeSH] OR "mycophenolic acid" [Title/Abstract] OR mycophenolate mofetil[Title/Abstract] OR rituximab[Title/Abstract] OR "azathioprine"[MeSH] OR azathioprine[Title/Abstract] OR immunosuppressant[Title/Abstract] OR immunomodulator [Title/Abstract]

EMBASE

'common variable immunodeficiency'/exp OR 'common variable immodeficiency':ab,ti,kw OR CVID:ab,ti,kw OR 'primary immunodeficienc*':ab,ti,kw OR 'antibody deficiency':ab,ti,kw OR GLILD:ab,ti,kw OR 'granulomatous lymphocytic interstitial lung disease'/exp OR 'granulomatous lymphocytic interstitial lung disease':ab,ti,kw OR ILD:ab,ti,kw OR 'granulomatous lung disease':ti,ab,kw OR 'interstitial lung disease':ab,ti,kw OR 'lymphocytic interstitial pneumonia':ti,ab,kw OR 'lymphocytic interstitial pneumonitis':ti,ab,kw OR 'lymphoid interstitial pneumonitis':ti,ab,kw

AND 'stem cell transplantation'/exp OR 'stem cell transplantation':ti,ab,kw OR 'hematopoietic stem cell transplantation':ti,ab,kw OR abatacept/exp OR abatacept:ab,ti,kw OR corticosteroid/exp OR corticosteroid:ab,ti,kw OR prednisone:ab,ti,kw OR 'mycophenolic acid'/exp OR 'mycophenolic acid':ti,ab,kw OR 'mycophenolate mofetil'/exp OR 'mycophenolate mofetil':ti,ab,kw OR methotrexate/exp OR methotrexate:ab,ti,kw OR immunosuppressant:ti,ab,kw OR immunomodulator:ab,ti,kw

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