Altered Spectrum of Lymphoid Neoplasms in a Single-Center Cohort of Common Variable Immunodeficiency with Immune Dysregulation

Claudia Wehr 1,2 · Leonora Houet 2 · Susanne Unger 2 · Gerhard Kindle 3,4 · Sigune Goldacker 2,5 · Bodo Grimbacher 3,6,7,8 · Andrés Caballero García de Oteyza 2 · Reinhard Marks 1 · Dietmar Pfeifer 1 · Alexandra Nieters 2,4 · Michele Proietti 2 · Klaus Warnatz 2,5 · Annette Schmitt-Graeff 9

Received: 29 April 2020 / Accepted: 2 March 2021 / Published online: 19 April 2021
© The Author(s) 2021

Abstract

Purpose Common variable immune deficiency (CVID) confers an increased risk of lymphoid neoplasms, but reports describing the precise WHO specification of the lymphoma subtypes and their immunological environment are lacking. We therefore classified lymphomas—occurring in a cohort of 21 adult CVID patients during a 17-year period at our center—according to the 2016 WHO classification and characterized the local and systemic immunological context

Results The median time between the onset of CVID and lymphoma was 14 years. Patients showed a high prevalence of preceding immune dysregulation: lymphadenopathy (n = 13, 62%), splenomegaly (n = 18, 86%), autoimmune cytopenia (n = 14, 67%), and gastrointestinal involvement (n = 15, 71%). The entities comprised extranodal marginal zone lymphoma (n = 6), diffuse large B cell lymphoma (n = 7), plasmablastic lymphoma (n = 1), classic Hodgkin lymphoma (n = 4, including three cases with germline CTLA4 mutations), T cell large granular lymphocytic leukemia (n = 2), and peripheral T cell lymphoma, not otherwise specified (n = 1), but no follicular lymphoma. An Epstein-Barr virus association was documented in eight of 16 investigated lymphomas. High expression of PDL1 by tumor cells in five and of PDL1 and PD1 by tumor-infiltrating macrophages and T cells in 12 of 12 investigated lymphomas suggested a tolerogenic immunological tumor environment.

Conclusion In summary, a diverse combination of specific factors like genetic background, chronic immune activation, viral trigger, and impaired immune surveillance contributes to the observed spectrum of lymphomas in CVID. In the future, targeted therapies, e.g., PD1/PDL1 inhibitors in CVID associated lymphomas with a tolerogenic environment may improve therapy outcome.

Keywords Common variable immunodeficiency (CVID) · lymphoma · Hodgkin lymphoma (HL) · diffuse large B cell lymphoma (DLBCL) · marginal zone lymphoma (MZL) · CTLA4
Introduction

Morbidity and mortality of CVID patients under immunoglobulin replacement are mainly determined by malignancies—lymphoid neoplasms and gastric adenocarcinoma—and clinical manifestations of immune dysregulation rather than infections [1–3]. The predisposition of CVID patients to develop lymphoid neoplasms has long been recognized [1, 2, 4–11]. Similar to other centers [8, 12, 13], 4% of our CVID patients developed lymphoma [14]. The timely detection and accurate diagnosis of a lymphoid neoplasm in CVID patients are both clinically and pathologically challenging. This can be due to pre-existing and concomitant lymphoid hyperplasia (long-standing lymphadenopathy, splenomegaly) and lack of biomarkers hamper to set the optimal time point for a biopsy. Additionally, the diagnosis of an overt malignant lymphoma and the assignment to a specific disease entity can pose significant challenges to the pathologist [15, 16]. A precise subclassification of immunodeficiency-associated lymphomas following the updated 2016 WHO system can be difficult as it was established for immunocompetent patients and immunodeficiency-associated lymphomas may present with pathologic variants. [17]. Boundaries between non-malignant lymphoproliferative disorders (LPD) and overt lymphomas are often difficult to recognize [18, 19]. Non-neoplastic LPDs may present with a profound modulation of the innate lymph node structure and contain morphologically abnormal lymphoid populations, including blast cells, especially in Epstein-Barr virus (EBV)-positive cases. On the other hand, abundant reactive immune infiltrates in the microenvironment of lymphomas can lead to misdiagnosis as a non-neoplastic lesion. Pitfalls also arise from an inadequate procurement of specimens, especially when samples are taken from the concomitant LPD and not from the neoplastic process. A multiparameter approach integrating routine and ancillary techniques as well as clinical information are mandatory for an appropriate diagnosis.

The mechanisms of lymphomagenesis in CVID are not completely understood. Higher IgM levels at diagnosis of CVID, female sex [1], a phenotype of late-onset combined immunodeficiency (IoCID) [20], polyclonal LPD [2, 12], and immune thrombocytopenia [13] have been associated with a higher risk in some cohorts; the latter however is not yet confirmed in the recent US registry report [12]. While increased risk for lymphoma development in CVID is broadly recognized, a precise categorization following the current WHO classification is only given for a subset of published lymphomas [11]. For a relevant number of cases, only a lineage assignment but no further definitive WHO classification is provided. Most publications report lymphomas of B cell origin, while T cell lymphomas are rare. Among the defined entities, the literature describes a predominance of extranodal marginal zone lymphoma (ENMZL) arising in the mucosa-associated lymphoid tissue (MALT) of the gastrointestinal tract, the salivary glands or in the bronchus-associated lymphoid tissue [4, 5]. But also classic Hodgkin lymphoma (CHL), diffuse large B cell lymphoma (DLBCL), and rare T cell neoplasms such as T cell large granular lymphocytic leukemia (T-LGLL) have been described [1, 2, 8, 20–22].

Thus, the characterization of the spectrum of CVID-associated lymphoid neoplasms and the biologic factors involved in their development warrant additional studies. To approach these questions, we retrospectively reviewed the clinical presentation, immunological phenotype, histologic features, and—if available—molecular abnormalities of 21 CVID-associated lymphoma cases collected from our institution.

Methods

Patient Cohort and Data Collection

Patients with CVID and histopathologically established diagnosis of lymphoid neoplasm were retrospectively identified. The diagnosis of CVID was based on ESID/PAGID criteria [23]. Patients with possible secondary immunodeficiency due to lymphoma or a concurrent diagnosis (<1 year between diagnosis of CVID and lymphoma) were excluded. The institutional review board approved the study according to the Declaration of Helsinki (No: 239/1999 and 121/11).

Targeted Next-Generation Sequencing of Germline Variants

Germline whole-exome sequencing (WES) data was available for 12 patients; one patient underwent targeted sequencing of the CTLA4 locus (Supplemental Methods).

Histopathological, Immunohistological, and Molecular Analyses of Tissue Specimens

The diagnosis of lymphoid neoplasm was based on the histopathological evaluation of tissue specimens and supported by conventional immunohistochemistry and molecular methods (Supplemental Methods). The large majority of cases were reevaluated according to the guidelines of the current revised 2016 WHO classification [16]. We further defined all tumor samples according to the unifying nomenclature for immunodeficiency-associated LPD [15]. DNA extracted from formalin-fixed paraffin-embedded tissue was available in six lymphomas and analyzed on an Illumina TruSight Lymphoid
Panel. Of the six samples, four were rejected due to low sample quality.

Results

Characteristics of the Patient Cohort

We identified 21 CVID patients with lymphoma (Table 1). Germline genetic disease-associated variants were proven in five of 13 analyzed patients: three CTLA4, one BACH2, and one TNFSR13B. Among 162 patients in the CVID cohort at our center who underwent genetic testing, 7.4% (n = 12) carried rare CTLA4 variants, 16% (n = 26) carried rare TNFSR13B variants, and 4.9% (n = 8) rare BACH2 variants. The median age of onset of first symptoms attributed to CVID was 29.5 years (interquartile range [IQR]: 21–38 years). This appeared lower in the subgroup with CTLA4 mutations (11, 15, 16 years). The median age at diagnosis of lymphoma was 38 years (IQR: 32–51 years). The median time between the onset of first symptoms attributed to CVID and diagnosis of lymphoid malignancy was 14 years (IQR 8–17 years); this was comparable in the subgroup of patients with CTLA4 mutations (15, 17, 18 years). Men were overrepresented in our cohort (male n = 15, female n = 7) consistent with the increased risk of lymphoma development in men in the general population [24].

High Prevalence of Immune Dysregulation and Immunosuppressive Treatment Preceding the Development of Lymphoid Neoplasm in CVID Patients

In the general CVID cohort, approximately one-third of CVID patients have non-infectious disease-related complications [2, 20, 25, 26]; however, in our lymphoma cohort, all patients suffered from preceding non-infectious disease-related complications. Consistent with the literature [2, 12], preceding lymphadenopathy (n = 13, 62%) or splenomegaly (n = 18, 86%) was highly prevalent in our cohort. Additionally, both autoimmune cytopenias (n = 14, 67%) and non-infectious gastrointestinal involvement (n = 15, 71%) were more prevalent in CVID patients developing lymphoma compared to the general CVID population (30% and 16%, respectively) [14]. Upon reevaluation of intestinal biopsies of CVID patients with lymphoma, we observed a variety of morphologic changes including ulcerative colitis-like colitis, celiac-like enteropathy, or microscopic enteritis with oligoclonal T cell expansion (Fig. 1). Due to the high prevalence of non-infectious inflammatory complications, 17 patients (81%) had received systemic immunosuppressive therapy before the diagnosis of lymphoma. Immunoglobulin levels and lymphocyte subpopulations were collected at diagnosis of CVID and before first lymphoma treatment. Even when accounting for 30% of missing values, the data indicated that the majority of studied patients (76%) had low IgM levels at diagnosis of CVID which was in contrast to previously published data [1, 2], and CD4 lymphocytopenia was more prevalent at diagnosis of lymphoma (n = 11, 52%) compared to initial diagnosis of CVID (n = 6, 29%). It remains an open question, whether the immunosuppressive treatment, the lymphoid neoplasm, the natural course of the immunodeficiency disorder, or a combination of these underlie the development of CD4 lymphocytopenia.

Detailed Characterization of Lymphoid Neoplasms

The following entities were diagnosed (Table 2): ENMZL (n = 5, 24%), splenic MZL (n = 1, 5%), DLBCL (n = 7, 33%), plasmablastic lymphoma (PBL, n = 1, 5%), mixed cellular CHL (MCCHL, n = 4, 19%), T-LGCL (n = 2, 10%), and nodal peripheral T cell lymphoma, not otherwise specified (PTCL, NOS, n = 1, 5%). According to the recently proposed unifying nomenclature for immunodeficiency-associated LPD [15], six cases were assigned to the category indolent B cell lymphoma while eight cases fulfilled criteria of aggressive B cell lymphoma. The lymphoid neoplasm was EBV associated in eight of 16 cases (50%), while an additional three cases (19%) only contained a low amount of small EBV+ bystander cells (Supplemental Table 1).

Indolent B Cell Lymphomas

The six indolent B cell lymphomas included four ENMZL of pulmonary MALT, one ENMZL of duodenal MALT, and one splenic MZL.

Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue

Two patients presented with Helicobacter pylori-negative ENMZL of the MALT of the upper gastrointestinal tract (patient 08, 16): Patient 08 was diagnosed with MZL in the gastric mucosa 16 years after diagnosis and complete remission of a pulmonary ENMZL (Fig. 2a–f). Whether the gastric ENMZL was a relapse or a second neoplasm could not be evaluated. The biopsy contained rare EBER+ small lymphocytes. Patient 16 presented with ENMZL in the duodenum and showed, besides lymphoepithelial lesions, a marked plasma cell differentiation. EBV was not tested.

De novo pulmonary ENMZL was diagnosed in three patients (patient 08, 15, 17), all suffering from recurrent respiratory tract infections (Fig. 2g–h). Two specimens were tested for EBV but were negative.
Table 1  Clinical characteristics of CVID patients with lymphoid neoplasms.

| Patient | Sex | IEI | Genetics (result) | MOI | ACMG | age at onset of symptoms of CVID | age at diagnosis of lymphoid neoplasm | lymphadenopathy | splenomegaly |
|---------|-----|-----|-------------------|-----|------|-------------------------------|--------------------------------------|-----------------|-------------|
| 1       | m   | CVID| BACH2, c.2362G>A  | AD  | BS1, BS2 strong, PP5 supporting| 42                             | 48                                           | 1               | 1           |
| 2       | f   | CVID| -                 | AD  | BS1, BS2 strong, PP5 supporting| 42                             | 52                                           | 0               | 1           |
| 3       | m   | CVID| -                 | AD  | BS1, BS2 strong, PP5 supporting| 35                             | 49                                           | 1               | 1           |
| 4       | f   | CVID| -                 | AD  | BS1, BS2 strong, PP5 supporting| 45                             | 48                                           | 1               | 1           |
| 5       | f   | CVID, evolved into loCID| CTLA4, c.531_544del, p.179fs | AD  | BS1, BS2 strong, PP5 supporting| 38                             | 49                                           | 1               | 1           |
| 6       | m   | CVID with CTLA4 mut.| TNFRSF13B, c.542C>A, p.A181E | AD  | BS1, BS2 strong, BP1, BP4 supporting, PM1 moderate, PM5, PP5 supporting| 30                             | 46                                           | 0               | 1           |
| 7       | m   | CVID| -                 | AD  | BS1, BS2 strong, BP1, BP4 supporting, PM1 moderate, PM5, PP5 supporting| 16                             | 33                                           | 1               | 1           |
| 8       | m   | CVID| -                 | AD  | BS1, BS2 strong, BP4, BP6 supporting| 31                             | 32                                           | 1               | 1           |
| 9       | m   | CVID| -                 | AD  | BS1, BS2 strong, BP4, BP6 supporting| 26                             | 38                                           | 1               | 1           |
| 10      | m   | CVID, evolved into loCID| CT2A c.223C>T, p.R75W | AD  | BS1, BS2 strong, BP1, BP4, BP6 supporting, PM1 moderate, PM5, PP5 supporting| 37                             | 38                                           | 1               | 1           |
| 11      | m   | CVID with CTLA4 mut.| -                 | AD  | BS1, BS2 strong, BP1, BP4, BP6 supporting, PM1 moderate, PM5, PP5 supporting| 11                             | 28                                           | 1               | 1           |
| 12      | m   | CVID (loCID)| no rare variants in PID associated genes identified| AD  | BS1, BS2 strong, BP1, BP4, BP6 supporting, PM1 moderate, PM5, PP5 supporting| 9                              | 29                                           | 1               | 1           |
| 13      | f   | CVID| -                 | AD  | BS1, BS2 strong, BP1, BP4, BP6 supporting, PM1 moderate, PM5, PP5 supporting| 21                             | 80                                           | 0               | 0           |
| 14      | f   | CVID| -                 | AD  | BS1, BS2 strong, BP1, BP4, BP6 supporting, PM1 moderate, PM5, PP5 supporting| 21                             | 80                                           | 0               | 0           |
| 15      | f   | CVID (loCID)| CVID STAT1 c.796G>A; p.V266I | AD  | BS1, BS2 strong, BP1, BP4, BP6 supporting, PM1 moderate, PM5, PP5 supporting| 47                             | 52                                           | 1               | 1           |
| 16      | m   | CVID, evolved into loCID| no rare variants in PID associated genes identified| AD  | BS1, BS2 strong, BP1, BP4, BP6 supporting, PM1 moderate, PM5, PP5 supporting| 27                             | 47                                           | 0               | 1           |
| 17      | f   | CVID (loCID)| no rare variants in PID associated genes identified| AD  | BS1, BS2 strong, BP1, BP4, BP6 supporting, PM1 moderate, PM5, PP5 supporting| 44                             | 52                                           | 0               | 1           |
| 18      | m   | CVID| no rare variants in PID associated genes identified| AD  | BS1, BS2 strong, BP1, BP4, BP6 supporting, PM1 moderate, PM5, PP5 supporting| 27                             | 46                                           | 1               | 1           |
| 19      | m   | CVID, evolved into loCID| no rare variants in PID associated genes identified| AD  | BS1, BS2 strong, BP1, BP4, BP6 supporting, PM1 moderate, PM5, PP5 supporting| 38                             | 52                                           | 0               | 1           |
| 20      | f   | CVID with CTLA4 mut.| CTLA4 c.165G>A, p.R55W | AD  | BS1, BS2 strong, BP1, BP4, BP6 supporting, PM1 moderate, PM5, PP5 supporting| 15                             | 30                                           | 0               | 0           |
| 21      | m   | CVID| no rare variants in PID associated genes identified| AD  | BS1, BS2 strong, BP1, BP4, BP6 supporting, PM1 moderate, PM5, PP5 supporting| 17                             | 34                                           | 1               | 0           |
| Patient | Inflammatory lung involvement | Autoimmune cytopenia (ITP/AIHA/AIN) | GI involvement | Other autoimmunity | Immunosuppression before onset of lymphoid neoplasm | Others | Duration of follow-up after diagnosis of lymphoid neoplasm until death or last visit |
|---------|------------------------------|--------------------------------------|----------------|-------------------|-----------------------------------------------------|--------|----------------------------------------------------------------------------------|
| 1       | 0                            | 1                                    | Lymphocytic colitis | AION              | Steroid                                             |        | 8 years                                                                           |
| 2       | 0                            | 1                                    | Lymphocytic duodenitis, colitis | 0                 | Steroid                                             |        | 1 month                                                                           |
| 3       | 0                            | 1                                    | 0                | 0                 | Steroid                                             |        | 3 years                                                                           |
| 4       | 0                            | 1                                    | Diarrhea and wasting (histology not available) | Neurodermitis, diabetes mellitus type I, autoimmune hepatitis | Steroid, Azathioprine                                  |        | 5 months                                                                           |
| 5       | 0                            | 1                                    | Seronegative enteropathy with features of refractory celiac disease and nodular lymphofollicular hyperplasia | Nodular regenerative hyperplasia of the liver | Steroid, Rituximab                                  |        | 2 years 1 month                                                                  |
| 6       | 0                            | 1                                    | Duodenal nodular lymphatic hyperplasia, lymphocytic ileitis | Lymphocytic encephalomyelitis | Steroid, Azathioprine                                  |        | 7 years 5 months                                                                  |
| 7       | 0                            | 1                                    | 0                | 0                 | Azathioprine                                        |        | Adenocarcinoma stomach                                                            | 23 years |
| 8       | 0                            | 1                                    | Seronegative enteropathy with features of refractory celiac disease and lymphofollicular hyperplasia | 0                 | 0                                                   |        | 13 years                                                                          |
| 9       | 0                            | 0                                    | 0                | 0                 | 0                                                   |        | 14 years 8 months                                                                 |
| 10      | 1                            | 0                                    | Ulcerative colitis-like enteropathy | 0                 | Steroid, Mesalazin | Recurrent CMV                                       |        | 5 years                                                                           |
| 11      | 0                            | 0                                    | 0                | Thyroiditis       | 0                                                   |        | 15 years                                                                          |
| 12      | 1                            | 1                                    | Seronegative enteropathy with features of refractory celiac disease | Interstitial nephritis | Steroid, Rituximab                                  |        | 2 years 8 months                                                                 |
| 13      | 0                            | 1                                    | Seronegative enteropathy with features of refractory celiac disease | Diabetes mellitus Type I, autoimmune hepatitis | Steroid                                               | CMV colitis | 7 months                                                                         |
| 14      | 0                            | 1                                    | 0                | 0                 | 0                                                   |        | 2 months                                                                          |
| 15      | 1                            | 0                                    | Nodular lymphofollicular hyperplasia (ileum) | 0                 | Steroid                                             |        | 2 years 2 months                                                                 |
| 16      | 1                            | 0                                    | Duodenitis, ileitis, cryptitis, chronic Campylobacter infection | Nodular regenerative hyperplasia of the liver, arthritis | Steroid, Sulfasalazine, Sirolimus                    |        | 1 year 8 months                                                                   |
| 17      | 1                            | 1                                    | Ileitis, colitis (ulcerative colitislike) | Nodular regenerative hyperplasia of the liver, arthritis | Steroid                                             |        | 12 years                                                                          |
| 18      | 0                            | 0                                    | Chronic diarrhea and wasting (histology not available) | Reactive arthritis | Sulfasalazine, Leflunomide, Hydroxychloroquine, Steroid |        | 9 years 2 months                                                                 |
| 19      | 0                            | 0                                    | 0                | 0                 | Steroid                                             |        | 5 years 7 months                                                                  |
Splenic Marginal Zone Lymphoma (SMZL)

Splenic MZL developed in patient 03 with preceding lymphoid hyperplasia and early lesions of EBV lymphoproliferation, follicular hyperplasia subtype (Fig. 3a–d). Splenectomy specimen showed a CD20+BCL2+IgM+IgD+ SMZL (Fig. 3e–f). A minority of small to large EBER+CD30+CD15− cells was present, predominantly associated with germinal centers (Fig. 3g–h) and was considered residual component of the preceding EBV lymphoproliferation [27]. The lymphoma was not classified as EBV + MZL as the dominant population was EBER negative.

In the MZL samples, tumor cells did not express PDL1, while moderate amounts of PDL1+ tumor-infiltrating cells (TICs), including macrophages and dendritic cells, and PD1+ tumor-infiltrating lymphocytes were present in the microenvironment (Fig. 6g–h, Supplemental Table 1).

Aggressive B Cell Lymphoma

The category of aggressive B cell lymphomas included seven DLBCL (four DLBCL, NOS; three EBV-positive DLBCL), one PBL, and four MCCHL.

Diffuse Large B Cell Lymphoma, Not Otherwise Specified (DLBCL, NOS)

DLBCL, NOS developed in patients 04, 05, 19, and 21. Three patients presented with predominantly nodal DLBCL. Patient 04 had widespread nodal and extranodal involvement secondary to an EBV-associated lesion composed of polymorphous B cells responding to rituximab, received for recurrent autoimmune hemolytic anemia. Patient 05 had a preceding history of polymorphic, polyclonal EBV-associated LPD in the liver and nodal MZL both resolving after rituximab therapy. The subsequent EBV-negative DLBCL expressed MND (myeloid cell nuclear differentiation antigen) in a subpopulation suggesting a derivation from the nodal MZL. Panel sequencing of the lymphoma showed mutations in MYD88 (p.Ser219Cys) and NOTCH2 (p.Gln2409Ter). Patient 21 had been treated for nodular lymphocyte-predominant Hodgkin lymphoma (NLPHL) 12 years earlier. Subsequent lymph node biopsies were consistent with complete remission of NLPHL and finally nodal DLBCL. No mutations were identified by lymphoma panel sequencing. Patient 19 presented with extranodal DLBCL involving the duodenum. EBER was not detected in patients 19 and 21, and only present in rare small intratumoral bystander lymphocytes in patient 05. It could not be tested in patient 04. According to the immunohistochemical cell of origin classification (COO) [16, 28], two DLBCL, NOS cases were of the germinal center B cell (GCB).
category (CD10 + IRF4/MUM1−; patient 20, 22) and two cases had a non-GCB phenotype (CD10-IRF4/MUM1+; patient 04, 05; Table 2).

PDL1 expression in tumor cells was detected in one (patient 05) out of three DLBCL, NOS cases by immunohistochemistry (Supplemental Table 1).

**EBV-Positive Diffuse Large B Cell Lymphoma**

This category includes three DLBCL cases in which EBER was detected in ≥80% of tumor cells (patient 01, 02, 09, Table 2, Supplemental Table 1). Patient 01 presented with extranodal DLBCL with multifocal involvement of the large bowel wall and the spleen (Fig. 4a–d), harboring a BCL6 translocation. The EBV latency type III (EBER+, EBNA2+, LMP1+) was similar to post-transplant lymphoproliferative disorders (Fig. 4e–h). Patient 02 had a predominant DLBCL manifestation in the massively enlarged spleen and MNDA expression in the lymphoma supported a derivation from splenic marginal zone cells [29]. A common histologic feature of both cases was the polymorphism of tumor cells including bizarre giant cells and areas of necrosis. The nodal LPD of patient 09 showed a T cell/histiocyte-rich background. All three EBV-positive DLBCL cases belonged to the non-GCB subcategory. Two cases in this category (patients 01, 02) were tested for PDL1 and PD1 expression. Both showed a strong positivity for PDL1 by most tumor cells and TICs (histiocytes/dendritic cells) as well as for PD1 in tumor-infiltrating lymphocytes (Fig. 6c–d, Supplemental Table 1).

**Plasmablastic Lymphoma**

Patient 13 was diagnosed with extranodal EBV+ PBL predominantly involving the rectal wall (Supplemental Fig. 1). The PBL showed a high proliferation fraction of 95%. Immunohistochemical staining suggested an EBV-latency type 1. EBV+ PBL has been reported to often express both PDL1 and PD1 [30]. In patient 13, the neoplastic population was PDL1 negative. The microenvironment contained rare PDL1+ TICs predominantly histiocytes/dendritic cells adjacent to tumor cells, but no PD1-positive cells (Fig. 6e, Supplemental Table 1).

**Classic Hodgkin Lymphoma**

MCCHL developed in four patients (patients 06, 12, 18, 20, Fig. 5). Germline CTLA4 mutations were detected in all tested patients (n = 3). The CHLs were assigned to EBV latency type II, which is characteristic for EBV + -CHL. EBV positivity was not restricted to the neoplastic population but also present in scattered small bystander cells. The microenvironment was abundantly composed of small T cells and large clusters of TICs, especially CD68+ histiocytes and protein S100 + CD11c + dendritic cells. The predominant T cell population expressed CD8 and cytotoxic molecules including granzyme B, perforin, and TIA. PDL1 was strongly expressed by tumor cells and TICs accompanied by abundant tumor-infiltrating PD1+ lymphocytes (Fig. 6a, Supplemental Table 1). Both PDL1- and PD1-positive cells were increased especially in the peripheral tumor tissue adjoining the surrounding non-neoplastic tissue.

**T Cell Lymphomas**

Three CVID patients were diagnosed with T cell neoplasms including two T-LGLL cases and one PTCL, NOS.

**T Cell Large Granular Lymphocytic Leukemia (T-LGLL)**

CD8+CD57+ T-LGLL developed in patients 10 and 11. T-LGLL did not only involve the peripheral blood and bone marrow but also infiltrated liver and colon (patient 10). Diagnosis was based on expansion of lymphocytes with typical LGL morphology and clonal rearrangement of the TCRγ chain. Bone marrow infiltration by LGL cells was associated with reticulin fibrosis grade two and maturation defects of the three hematopoietic lineages (Supplemental Fig. 2) resulting in transfusion dependency. EBV testing of histologic specimens could not be performed.

**Peripheral T Cell Lymphoma, Not Otherwise Specified (PTCL, NOS)**

Patient 14 had extensive nodal involvement by an EBV-negative PTCL, NOS at the age of 80 years after a lifelong course of an “infection only” CVID complicated by one single episode of ITP. Considering the rarity of T cell lymphomas described in CVID and the advanced age of the patient at diagnosis of the PTCL, the PTCL might not be a complication of the immunodeficiency but rather a co-incidence of two different diseases.

**Clinical Outcome in CVID Patients with Lymphoid Neoplasm**

All patients with indolent B cell lymphoma (n = 6) and MCCHL (n = 4) were treated with immuno-chemotherapy or rituximab alone, and were alive at data retrieval. Patient 03 underwent allogeneic hematopoietic cell transplantation (alloHCT). The median duration of follow-up for indolent B cell lymphoma was 7.5 years (IQR 2.4–11.3) and for MCCHL 5 years (IQR 2.1–6). Of the patients with aggressive B cell lymphomas, four of eight patients were alive and in remission at data retrieval (median: 3.8 years, total range 0.7–14.7). Two of these patients underwent alloHCT. Four patients with
aggressive B cell lymphomas died from either progression ($n = 3$) or neutropenic fever ($n = 1$); median time until death was 0.5 years (total range 0.1–2.1). One patient with T-LGLL died from progressive disease 5 years after diagnosis, and another was cured by alloHCT and in complete remission for >10 years at data retrieval. In summary, with the restrictions of a retrospective chart review and the limited number of patients, we conclude that the prognosis was predominantly determined by the underlying lymphoid neoplasm, while treatment toxicity and infections had a minor impact.

**Discussion**

To our knowledge, this study summarizes the largest analysis of lymphoid neoplasms categorized according to the updated 2016 WHO classification [16] in patients diagnosed with CVID. Lymphoid malignancies developed at a younger median age in our cohort (38 years, IQR: 32–51) compared to the median age at lymphoma diagnosis in the general UK population (67.2 years, IQR: 54.9–76.5) [24]. The median latency of 14 years between the onset of CVID and diagnosis of lymphoma may not reflect the true latency since we had excluded lymphoma diagnosed before or within the first year of CVID diagnosis. The histopathologic review identified DLBCL (33%), MZL (29%), and MCCHL (19%) as the three most prevalent lymphoma subtypes. Among the MZL, five cases were ENMZL of MALT ($n = 5$, 24%) and one splenic MZL ($n = 1$, 5%). In the general UK population, DLBCL represented 40.9%, CHL 12.7%, and all MZL subtypes 17%. Importantly, ENMZL represented only 3.6% of lymphoid neoplasms in the general population [24] highlighting that MZL, with the caveat of statistics based on small numbers, is highly overrepresented in our and previous series [4,5]. From the literature, we retrieved 53 B cell lymphomas classified according to the current nomenclature (Supplemental Table 2). Among these, MZL represented 32% and HL 30% of cases comparable to 33% and 22% of B cell lymphomas in our cohort. The prevalence of DLBCL (39%) was higher in our cohort compared to the literature (21%). Noteworthy, many B cell malignancies diagnosed in CVID, such as DLBCL, HL, and MZL, result from the malignant transformation of mature B cells that have experienced the germinal center reaction and usually carry somatic mutations.
mutations of immunoglobulin genes but not from unmutated B cell populations. To our knowledge, mantle cell lymphomas that predominantly derive from mature B cells that do not enter the follicular germinal center and carry no or a limited number of \( IGHV \) somatic mutations are not reported among the CVID-associated lymphoid neoplasms. Follicular lymphoma, in which hypermutation and class switching occur early in the lymphoma development, is uncommon in CVID (3 of 53 cases [11, 13, 31, 32]). It was also not present in our cohort despite its prevalence of 16% in the general population [24].

Mechanisms increasing the risk for lymphoid neoplasms in immunodeficiency include chronic inflammation and immune stimulation, transforming viral events, decreased immunological tumor surveillance, and other host factors including genetics [33, 34]. These risk factors might differ between lymphoma subtypes. In line with this hypothesis, we found a high incidence of non-infectious, inflammatory complications and non-malignant LPD preceding the malignancy. In addition to the previously reported increased risk of developing lymphoma in patients with splenomegaly, lymphadenopathy [2, 12], and autoimmune cytopenia [13], we also found a higher prevalence of gastrointestinal complications in CVID lymphoma patients compared to the general CVID population. Inflammation caused by chronic antigenic stimulation, due to recurrent infections or autoimmune diseases at mucosal sites, induces the development of organized lymphoid tissue in EN sites. Clonal expansion of MZ cells leads to the development of MZL of MALT [35, 36], which represents the second most frequent B cell lymphoma subtype in our cohort. In three of four CVID patients, the primary site of MZL involvement was the lung, despite the association with chronic gastrointestinal inflammation. In fact, in the general population, the gastrointestinal tract is most often affected [37].

**Table 2** Key pathological findings in CVID patients with lymphoid neoplasms.

| Patient | Classification of lymphoid neoplasm according to WHO classification 2016* | Hans classifier | EBV status lymphoma | Assessment of clonality status # |
|---------|-------------------------------------------------|-----------------|---------------------|----------------------------------|
| 1       | EBV+ DLBCL                                      | non-GCB         | +                   | clonal IgH gene rearrangement    |
| 2       | EBV+ DLBCL                                      | non-GCB         | +                   | clonal IgH gene rearrangement    |
| 3       | splenic MZL                                     | non-GCB         | -                   | clonal IgH gene rearrangement    |
| 4       | DLBCL, NOS                                      | non-GCB         | -                   | clonal IgH gene rearrangement    |
| 5       | DLBCL, NOS with preceding nodal MZL             | non-GCB         | -                   | clonal IgH gene rearrangement    |
| 6       | MCCHL                                           | non-GCB         | +                   | clonal IgH gene rearrangement    |
| 7       | extranodal MZL of MALT type (lung, stomach)     | non-GCB         | -                   | clonal IgH gene rearrangement    |
| 8       | extranodal MZL of MALT type (lung)              | non-GCB         | -                   | clonal IgH gene rearrangement    |
| 9       | EBV+ DLBCL                                      | non-GCB         | +                   | clonal IgH gene rearrangement    |
| 10      | T-LGLL                                          | unknown         | clonal TCRgamma gene rearrangement |
| 11      | T-LGLL                                          | unknown         | clonal TCRgamma gene rearrangement |
| 12      | MCCHL                                           | +               | no rearranged IgH chain genes |
| 13      | plasmablastic lymphoma                          | non-GCB         | +                   | clonal IgH gene rearrangement    |
| 14      | PTCL, NOS                                       | unknown         | clonal TCRgamma gene rearrangement |
| 15      | extranodal MZL of MALT type (lung)              | -               | clonal IgH gene rearrangement. |
| 16      | extranodal MZL of MALT type (duodenum)          | unknown         | clonal IgH gene rearrangement |
| 17      | extranodal MZL of MALT type (lung)              | unknown         | clonal IgH gene rearrangement. |
| 18      | MCCHL                                           | +               | no rearranged IgH chain genes |
| 19      | DLBCL, NOS (duodenum)                           | GCB             | -                   | clonal IgH gene rearrangement    |
| 20      | MCCHL                                           | +               | no rearranged IgH chain genes |
| 21      | DLBCL, NOS with preceding nodular lymphocyte predominant HD | GCB | - | clonal IgH gene rearrangement |

#Assessment of clonality status by PCR using BIOMED-2 primers. Abbreviations: CVID: common variable immunodeficiency, EBV:Epstein-Barr virus, DLBCL: diffuse large B-cell lymphoma, NOS: not otherwise specified, MCCHL: Mixed cellularity classic Hodgkin lymphoma, MZL: marginal zone lymphoma, MALT: mucosa-associated lymphoid tissue, T-LGLL: T-cell large granular lymphocytic leukemia, PTCL: Peripheral T cell lymphoma, DLBCL: diffuse large B cell lymphoma, MZL: marginal zone lymphoma, MALT: mucosa-associated lymphatic tissue. 1: present, 0: absent, n.a.: not available.
cases even after a long latency period [16]. A potential transformation of MZL into DLBCL as part of the newly characterized C1 cluster of DLBCL [38] may underlie the lymphoma in patient 05, as it carried a mutation of NOTCH2 that belongs to the frequently affected genes in splenic and nodal MZL. This hypothesis is also supported by expression of MNDa protein, however due to lack of material could not be molecularly confirmed. In addition, the DLBCL of patient 05 had a MYD88<sup>non-L265P</sup> mutation that was enriched in the molecularly defined C1 cluster of DLBCL [38]. This cluster frequently harbors NOTCH2 mutations, predominantly includes ABC-type tumors, and exhibits multiple bases of immune escape [38]. According to the COO designation for DLBCL, only two of seven cases in our cohort were of the GCB subgroup, while the remainder belonged to the non-GCB subgroup. These included the three EBV-associated DLBCLs. One GCB-type DLBCL contained no mutation by NGS analysis. No reliable data highlighting the genetic landscape and molecular pathogenesis of DLBCL in the context of CVID is currently available.

The link between EBV and tumor development in immunocompromised patients is well established [39]. We detected
an EBV association in eight of 16 cases including DLBCL, PBL, and MCCHL. However, other classical EBV-associated malignancies (nasopharyngeal carcinoma, uncommon B cell lymphoma entities, T/NK-LPD/lymphomas, Burkitt lymphoma) were not present. The proportion of EBV-associated DLBCL was higher (43%) in our cohort compared to the general Western population (<5% of cases) [16, 40]. EBV+ DLBCLs are classified as a separate entity, often of an ABC subtype and express PDL1 on their surface. These lymphomas are more frequently associated with decreased tumor surveillance in immunodeficiency [16, 41–43]. Accordingly, the EBV+ DLBCLs in our cohort were of non-GCB type and showed PDL1 expression in tumor cells, supporting a tolerogenic environment. The four cases of CHL, all being of MCCHL subtype, were EBV positive and showed a tolerogenic environment with PDL1 and PD1 expression, especially in the peripheral areas of the tumor tissue. MCCHL is typically associated with human immunodeficiency virus infection [44] and is characterized by a dense infiltrate of non-malignant immune cells including CD4+, CD8+, and regulatory T cells [45]. Remarkably, three of four patients with MCCHL carried germline CTLA4 mutations. The EBV-association rate for CHL in the general population of Western countries is about 30–35%, but for MCCHL, it is as high as 75% [16, 39]. The
The genetic background of CTLA4 mutations may play an important role in the surveillance of EBV-driven transformation and the predisposition to MCCHL. Reduced CTLA4 expression on tumor-infiltrating T cells might permit increased expression of CD80/CD86 on Hodgkin-Reed-Sternberg cells, thereby increasing T cell proliferation and modifying the tumor environment.

Lymphomas of T cell lineage are reported in a small number of CVID patients. Riaz et al. [11] included nine cases and the Czech nation-wide study two cases [13]. We diagnosed a T-LGLL in two patients and a PTCL, NOS in an additional patient. In CVID patients, increased numbers of polyclonal LGLs associated with neutropenia have been reported [21], and in one case, granulomatous lymphocytic interstitial lung disease was detected [22]. Potentially triggered by a chronic persistent stimulus, T-LGLL carries somatic STAT3 mutations in about 30–40% of cases and is often associated with autoimmune disorders [16, 46]. Germline gain-of-function mutations in STAT3 have been identified in some CVID-like patients [47]. The STAT3 mutational status of our T-LGLL patients is unknown; however, both had preceding lymphoid hyperplasia and autoimmune phenomena supporting the concept of an underlying inflammatory condition.
While it is attractive to speculate that the reduction of the discussed predisposing risk factors is a reasonable approach to reduce the lymphoma burden in CVID, there is insufficient data to conclude if and how non-malignant LPD and chronic inflammation can be successfully treated in the context of CVID and if this will reduce the risk of a lymphoid malignancy. On the other hand, immunosuppression might even elevate the risk of lymphoma development as it has been shown for the use of thiopurines and/or anti-TNF treatment in inflammatory bowel disease [48].

Currently, the treatment of lymphoid neoplasms is following treatment guidelines for immunocompetent patients [33]. Specific findings in immunodeficiency-associated lymphomas may be relevant weighing the different therapeutic options. The high percentage of EBV association and evidence of a tolerogenic tumor environment in CVID-associated DLBCL and CHL suggest that they may be candidates for immune checkpoint inhibitor therapy. The potential risks and benefits of checkpoint inhibitor therapy in patients with immune dysregulation have to be evaluated individually. In general, prognosis and treatment of B/T cell lymphomas depend on subtype, risk factors, and tumor stage at diagnosis. Meaningful tumor staging is often difficult in CVID patients due to preceding lymphoid hyperplasia, raising concern for comparisons to the general population’s outcome data. Nonetheless, even when considering stage IV disease for all
CVID patients, a mortality rate of 50% in DLBCL patients after 6 months is high compared to the general population and the German HIV-related lymphoma cohort [49]. Thus, given the early onset of lymphoid neoplasms in CVID, the dismal prognosis, and the genetic predisposition in some of the patients, we suggest to consider alloHCT in first remission [50, 51].

Overall, our data supports that autoimmune manifestations and non-malignant LPD are risk factors for the development of lymphoid malignancies in CVID. The spectrum of lymphomas differs in several aspects from the general population and most likely reflects the underlying immunodeficiency as a pathogenetically relevant factor. In the majority of patients, the exact risk factors and mechanisms initiating tumorigenesis and promoting progression to overt lymphoma still remain elusive and may be linked to different molecular subgroups. In the future, international collaborative efforts will have to shed light into these mechanisms of lymphomagenesis in CVID. Multicenter trial designs should include clinically and genetically defined patients, a central review of non-malignant and malignant LPD and the molecular landscape of tumor samples and preceding non-malignant lesions.
Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10875-021-01016-4.

Abbreviations alloHCT, Allogeneic hematopoietic cell transplantation; CHL, Classic Hodgkin lymphoma; CVID, Common variable immunodeficiency; DLBCL, Diffuse large B cell lymphoma; EBV, Epstein-Barr virus; ENMZL, Extranodal marginal zone lymphoma; loCJD, Late-onset combined immunodeficiency; LPD, Lymphoproliferative disorders; MALT, Mucosa-associated lymphoid tissue; MCCHL, Mixed-cellularity classic Hodgkin lymphoma; MNDa, Myelodysplastic cell nuclear differentiation antigen; MZL, Marginal zone lymphoma; NLPHL, Nodular lymphocyte-predominant Hodgkin lymphoma; PBL, Plasmablastic lymphoma; PTCL, NOS, Peripheral T cell lymphoma, not otherwise specified; SMZL, Splenic marginal zone lymphoma; T-LGCL, T cell large granular lymphocytic leukemia; TICs, Tumor-infiltrating cells; WES, Whole-exome sequencing

Acknowledgements We thank Marion Klima and Monika Erler for nursing care, Baerbel Weinhold, Katja Graewe and Beate Vollmer-Kary for excellent technical assistance, Kathryn Payne for proof-reading and all patients for the willingness to share their data.

Author Contributions Statement CW, LH, ASG, and KW planned the study. CW, SU, and LH extracted clinical data from charts. GK and AN extracted clinical data from the database. ASG performed the precise diagnostic evaluation of lymphoma samples. SU, SG, RM, and BG provided patient data. MP, AO, and BG provided genetic data. CW, LH, KW, and ASG wrote the manuscript. All authors approved the content of the manuscript.

Funding Open Access funding enabled and organized by Projekt DEAL. This study was supported by the German Federal Ministry of Education and Research [BMBF 01EO1305].

Declarations

Conflict of Interest The authors declare no competing interests.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, and indicated if changes were made. The images or other third party material in this article are included in the article’s Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

References

1. Resnick ES, Mosher EL, Godbold JH, Cunningham-Rundles C. Morbidity and mortality in common variable immune deficiency over 4 decades. Blood. 2012;119:1650–7.
2. Chapel H, Lucas M, Lee M, Bjorkander J, Webster D, Grimbacher B, et al. Common variable immunodeficiency disorders: division into distinct clinical phenotypes. Blood. 2008;112:277–86.
3. Pulvirenti F, Pecoraro A, Cinetto F, Milito C, Valente M, Santangeli E, et al. Gastric Cancer Is the Leading Cause of Death in Italian Adult Patients With Common Variable Immunodeficiency. Front Immunol. 2018;9:2546.
4. Cunningham-Rundles C, Cooper DL, Duffy TP, Strauchen J. Lymphomas of mucosal-associated lymphoid tissue in common variable immunodeficiency. Am J Hematol. 2002;69:171–8.
5. Aghamohammadi A, Parvaneh N, Tigran F, Mahjoob F, Movahedi M, Ghargrozolou M, et al. Lymphoma of mucosa-associated lymphoid tissue in common variable immunodeficiency. Leuk Lymphoma. 2006;47:343–6.
6. Kinlen LJ, Webster AD, Bird AG, Haile R, Peto J, Soothill JF, et al. P r o s p e c t i v e s t u d y o f c a n c e r i n p a t i e n t s w i t h hypogammaglobulinemia. Lancet. 1985;1:263–6.
7. Vorechovsky I, Litzman J, Lokaj J, Hausner P, Poch T. Common variable immunodeficiency and malignancy: a report of two cases and possible explanation for the association. Cancer Immunol Immunother. 1990;31:250–4.
8. Mayor PC, Eng KH, Singel KL, Abrams SI, Odunsi K, Moysich KB, et al. Cancer in primary immunodeficiency diseases: Cancer incidence in the United States Immune Deficiency Network Registry. J Allergy Clin Immunol. 2018;141:1028–35.
9. Jonkman-Berk BM, van den Berg JM, Ten Berge IJM, Bredius RGM, Driessen GJ, Dal M, et al. Primary immunodeficiencies in the Netherlands: national patient data demonstrate the increased risk of malignancy. Clin Immunol. 2015;156:154–62.
10. Ondoletkova I, Kindle G, Quinti I, Grimbacher B, Knerr V, Gathmann B, et al. The burden of common variable immunodeficiency disorders: a retrospective analysis of the European Society for Immunodeficiency (ESID) registry data. Orphanet J Rare Dis. 2018;13:201.
11. Riaz IB, Faridi W, Patnaik MM, Abraham RS. A systematic review on predisposition to lymphoid (B and T cell) neoplasias in patients with primary immunodeficiencies and immune dysregulatory disorders (inborn errors of immunity). Front Immunol. 2019;10:777.
12. Yakaboski E, Fuleihan RL, Sullivan KE, Cunningham-Rundles C, et al. Lymphoid proliferative disease in CVID: a report of types and frequencies from a US patient registry. J Clin Immunol. 2020. https://doi.org/10.1007/s10875-020-00769-8.
13. Kralickova P, Milota T, Litzman J, Malkusova I, Jilek D, Petanova EBocic, et al. Cancer in primary immunodeficiency disorders: unusual case studies and immunohistological retrospective analysis of types and possible explanation for the association. Cancer Immunol Immunother. 1990;31:250–4.
14. Mayer PC, Eng KH, Singel KL, Abrams SI, Odunsi K, Moysich KB, et al. Cancer in primary immunodeficiency diseases: Cancer incidence in the United States Immune Deficiency Network Registry. J Allergy Clin Immunol. 2018;141:1028–35.
15. von Spee-Mayer C, Koenn M, Wyhr C, Goidacke S, Kindle G, Bulashevskova A, et al. Evaluating laboratory criteria for combined immunodeficiency in adult patients diagnosed with common variable immunodeficiency. Clin Immunol. 2019;203:59–62.
16. Natkunam Y, Gratzinger D, Chadburn A, Goodlad JR, Chan JKC, Said J, et al. Immunodeficiency-associated lymphoproliferative disorders: time for reappraisal? Blood. 2018;132:1871–8.
17. Swedlow SH, Campo E, Harris NL, Pileri SA, Jaffe ES, Stein H, et al., editors. WHO classification of tumours of haematopoietic and lymphoid tissues. 4th ed. International Agency for Research on Cancer Volume 2; 2017.
18. de Jong D, Roemer MGM, Chan JKC, Goodlad J, Gratzinger D, Chadburn A, et al. B-cell and classical Hodgkin lymphomas associated with immunodeficiency: 2015 SH/EAHP workshop report-part 2. Am J Clin Pathol. 2017;147:153–70.
19. da Silva SP, Resnick E, Lucas M, Lortan J, Patel S, Cunningham-Rundles C, et al. Lymphoid proliferations of indeterminate malignant potential arising in adults with common variable immunodeficiency disorders: unusual case studies and immunohistological review in the light of possible causative events. J Clin Immunol. 2011;31:784–91.
20. Natkunam Y, Gratzinger D, de Jong D, Chadburn A, Goodlad JR, Chan JKC, et al. Immunodeficiency and dysregulation: report of the 2015 workshop of the society for hematopathology/European association for haematopathology. Am J Clin Pathol. 2017;147:124–8.
