Original Research

Turk J Immunol 2023;11(1):29-36
DOI: 10.4274/tji.galenos.2023.36854

Classification of Common Variable Immunodeficiency Using Measurement of B-cell Subsets in Moroccan Patients

Khaoula Mokhantar[1], Abir Allaoui[1,2], Fatima Ailal[1,3], Jalila El Bakkouri[1,4], Kaoutar Ouazahrou[1], Abderrahmane Errami[1], Ahmed Aziz Bousfiha[1,3], Mina Moudatir[1,5]

[1]Laboratory of Clinical Immunology, Inflammation, and Allergy (LICIA), Faculty of Medicine and Pharmacy, Hassan II University, Casablanca, Morocco
[2]Department of Internal Medicine, Cheikh Khalifa International University Hospital, Mohammed VI University of Health Sciences, Casablanca, Morocco
[3]Department of Pediatric Infectious and Immunological Diseases, Abderrahim El Harouchi Mother-Child Hospital, University Hospital Center Ibn Rochd, Hassan II University, Casablanca, Morocco
[4]Immunology Laboratory, University Hospital Center Ibn Rochd, Hassan II University, Casablanca, Morocco
[5]Department of Internal Medicine, University Hospital Center Ibn Rochd, Hassan II University, Casablanca, Morocco

Cite as: Mokhantar K, Allaoui A, Ailal F, El Bakkouri J, Ouazahrou K, Errami A, Bousfiha AA, Moudatir M. Classification of Common Variable Immunodeficiency Using Measurement of B-cell Subsets in Moroccan Patients. Turk J Immunol 2023;11(1):29-36

Received: 20.11.2022 **Accepted:** 27.02.2023

Corresponding Author: Khaoula Mokhantar, Laboratory of Clinical Immunology, Inflammation, and Allergy (LICIA), Faculty of Medicine and Pharmacy, Hassan II University, Casablanca, Morocco
E-mail: khaoulamokhantar@gmail.com **ORCID:** orcid.org/0000-0003-4451-2780

Abstract

Objective: Common variable immunodeficiency (CVID) is a complex inborn error of humoral immunity with complications of infectious and non-infectious origins. Classifications of CVID patients provide a clearer understanding of the pathogenesis, prediction, and management of non-infectious complications. This study aimed to classify Moroccan CVID patients using B-cell immunophenotyping, based on the European classification (EUROclass).

Materials and Methods: We recruited 20 CVID patients fullfilling established diagnostic standards. After collecting clinical and demographic data, we analyzed B-cell subsets by flow cytometry, grouped patients, and assessed the relationship of each group with clinical manifestations.

Results: In our cohort, 90% of the patients had a clinical history of respiratory infections. The non-infectious manifestations included splenomegaly, autoimmunity, lymphadenopathy, and granulomatous diseases diagnosed in 50%, 45%, 40%, and 25% of patients, respectively. We observed significant co-occurrence of splenomegaly with autoimmunity and to a lesser extent with granulomatous diseases. Patients had a significant reduction in total, switched memory, marginal zone-like, and plasmablasts, along with a strong increase in the percentage of activated B-cells, suggesting a defect in the late phases of B-cell differentiation. This condition was linked with an increased occurrence of splenomegaly and granulomatous affections. Besides, patients had also an expansion of $CD21^{low}$ B-cells, which was strongly associated with splenomegaly.

Conclusion: The classification of the first Moroccan cohort of CVID patients showed agreement with previous results. It suggests the possibility of adopting this approach on a global scale for better diagnosis and follow-up of CVID patients.

Keywords: B-cell subsets, common variable immunodeficiency, flow cytometry, hypogammaglobulinemia

Introduction

Common variable immunodeficiency (CVID) is a heterogeneous inborn defect of humoral immunity characterized by hypogammaglobulinemia caused by defective B-cell function. This defect is associated with high susceptibility to recurrent pyogenic infections, gastrointestinal disease, lymphoproliferative disorders, autoimmune manifestations, and malignancy (1-3). With a prevalence of 1 in 25.000 in the general population, CVID is the most frequently reported symptomatic inborn error of immunity (IEI), especially in adults, accounting for 30-70% of all identified patients with IEIs (4,5). While the highest prevalence of CVID among IEI/primary immunodeficiency diseases (PID) patients is documented in the United States at 40.2%, the lowest prevalence is observed in Middle Eastern and African countries with only 2.6% and 1.3%

ORCID: K. Mokhantar 0000-0003-4451-2780, A. Allaoui 0000-0002-5715-6300, F. Ailal 0000-0001-5824-3193, J. E. Bakkouri 0000-0003-2303-3369, A. Errami 0000-0002-2916-5007, A. A. Bousfiha 0000-0002-5011-9873

of PID patients, respectively (6). This is due to inequality in diagnosis, registration, and case reporting (7). The broad clinical and immunological phenotype of CVID leads to difficulties and delays in diagnosis, prompting the establishment of local or global classifications and guidelines based on clinical manifestations and laboratory data.

The first classification of CVID was proposed by Warnatz et al. (8) in 2002, it divides CVID patients into two main groups based on switched memory B cells (groups I and II) and two subgroups based on $CD21^{low}$ B-cells numbers (groups Ia and Ib). Then, the Paris classification based only on memory B-cell populations was suggested by Piqueras et al. (9), which classified patients into three groups (MB0, MB1, and MB2). Later in 2008, the EUROclass was established by Wehr et al. (10), in which CVID patients are subdivided based on transitional B-cell populations, switched memory, and $CD21^{low}$ B-cells. In the EUROclass, patients are divided into two main groups, those with a percentage of CD19 B-cells greater than >1% constitute group B^+, and patients with less than or equal to ≤1% of B-cells are in group B^-. Group B^+ is then divided into two groups based on the proportion of switched memory B-cells: Group smB^+, in which patients have more than >2% of switched memory B-cells, and Group smB^-, which consists of patients with ≤2% of switched memory B-cells (10). The smB^- group patients are then divided depending on the proportion of transitional B-cells into two subgroups, namely group smB-Tr^{hi}, which has >9% of transitional B-cells, and group smB- Tr^{norm}, which has less than 9% of transitional B-cells. Furthermore, the EUROclass differentiates patients of groups smB^- and smB^+ based on the expansion of $CD21^{low}$ B-cells into two groups; those admitted as group $CD21^{low}$ have ≥10% of $CD21^{low}$ B-cells, whereas patients classified as group $CD21^{norm}$ have <10% of $CD21^{low}$ B-cells (10,11). A fourth classification that categorizes patients into five different phenotypes based on the B-cell subset maturation was later proposed (12).

The need to classify patients with CVID aims to identify more homogeneous groups in order to establish common clinical and/or biological traits. These groups will also aim to restrict the field of investigation in an attempt to characterize the genetic defects specific to each group. Moreover, the importance of these classifications lies in the prediction of non-infectious complications in each group. For example, autoimmune manifestations occur more frequently in patients in groups MB0 and MB1, compared to patients in group MB2, while an increased granulomatous disease and splenomegaly are recorded in group MB0, based on the Paris classification (9). According to the EUROclass, splenomegaly and granulomatous affections are more likely to occur in patients with substantially diminished switched memory B-cells. In addition, the expansion of $CD21^{low}$ B-cells is linked with splenomegaly, while transitional B-cell augmentation is significantly correlated with the occurrence of lymphadenopathies (10,11). Furthermore, the EUROclass classification helped to define the groups of CVID patients with adequate or poor vaccine response (13). In this study, we will consider the first classification of Moroccan CVID patients, regarding their particular genetic background, based on the EUROclass classification by phenotyping B-cell subpopulations.

Materials and Methods

Patients

Over a period of 3 years (2017-2020), we included all CVID patients diagnosed within the Clinical Immunology Unit and the Department of Internal Medicine of Ibn Rochd University Hospital of Casablanca. In our department, we adopted the European Society for Immunodeficiencies/Pan-American Group for Immune Deficiency CVID diagnostic criteria, which include a significant decrease of immunoglobulin G (Ig) (at least 2 standard deviations below the reference value), as well as a significant decrease in at least one isotype IgA or IgM, in patients over the age of 4 years, with an onset of clinically considerable immunodeficiency and without any other causes of hypogammaglobulinemia (14). Only patients diagnosed after 2016 were tested for impaired vaccine responses or the absence of isohemagglutinins (14). All patients were regularly treated with subcutaneous/intravenous Ig substitution, but the blood samples were always collected before the treatment. B-cell phenotyping was performed through flow cytometry in patients at the age of ≥4 years and with a peripheral B-cells count over 1% of circulating lymphocytes. Three patients with less than 1% of B-cells were excluded from further flow cytometric analysis. This study received ethical committee clearance (Centre Hospitalier Universitaire Ibn Rochd; approval number: 220/DOEHRS1/013) and written informed consent signed by patients or their parents was accomplished.

Data Collection

For each patient, a standardized form was developed, completed, and validated by a CVID expert. Patients' data included personal information, clinical features (splenomegaly, granulomatous disease, lymphadenopathy, and autoimmune disorders anemia, thrombocytopenia, thyroiditis, vitiligo, arthritis, and others) as well as their immunological results.

Immunophenotyping Via Flow Cytometry

Flow cytometry investigation was carried out on fresh peripheral blood lymphocytes from patients with >1% of circulating B-cells (3.3% to 22.7%). Blood samples were

obtained from patients before intravenous Ig substitution and from age-matched controls and prepared as follows: The whole blood is stained with a variety of antibodies at appropriate concentrations for 20 minutes at 4°C: anti-CD19 phycoerythrin (PE-Cy7), anti-CD27-V500, anti-CD38 allophycocyanin (APC-H7), anti-CD21-V450, anti-IgD-PE, and anti-IgM fluorescein isothiocyanate (all from BD Biosciences). Four-color data acquisition was performed with FACSCanto-II automate and examined with FACSDiva 8.0.1 analysis software (both from BD Biosciences). Isotype control antibodies (all from BD Biosciences) were used to distinguish non-specific background staining from the specific antibodies staining.

Statistical Analysis

The SPSS Statistics for Windows (version 14) was used for all the statistical analyses. Depending on the distribution of data, the following tests were performed: Mann-Whitney U test, independent-samples t-test, paired-samples t-test, Wilcoxon signed ranks test, Pearson correlation test, or Spearman ranks.

Results

Demographic and Clinical Characteristics

Our study involved 20 CVID patients (10 males and 10 females) from 19 different Moroccan families, including one case of fraternity. The median age of the studied patients was 25.5 years (± 14.23 years). The mean age at diagnosis was 18.50 years (± 13.8 years) while the median age at onset was 13.85 years (± 12.92 years). The mean age at onset and diagnosis was higher in females (11.5 and 15.5 years) compared to males (7.5 and 10.5 years); however, there was no difference in the delay of diagnosis between both genders (3.5 years). Twelve patients (60%) were born to consanguineous parents. Eighteen patients (90%) had a history of respiratory infections. Other clinical manifestations included splenomegaly in ten patients (50%), with no difference in the incidence between males and females; autoimmunity in nine patients (45%), which was slightly higher in males (5/9); and lymphadenopathy in eight patients (40%), which was higher in females (5/8). Granulomatous diseases were diagnosed in five cases (25%), without significant differences in sex (Table 1).

Cooccurrence and association of splenomegaly, granulomatous diseases, and autoimmune manifestations have been illustrated in our cohort of patients (Figure 1). Autoimmune manifestations were significantly associated with splenomegaly (p=0.004), and 66.6% (6/9) of patients with autoimmune manifestations had splenomegaly. In addition, splenomegaly was significantly associated with granulomatous diseases (p=0.017), and more than half of patients with granuloma (3/5) also had splenomegaly. There was no statistically significant association of autoimmunity with granulomatous manifestations in our cohort (p=0.291) and only one of the patients with autoimmunity (1/9) had a granulomatous disease. The association of lymphadenopathy with other manifestations was not significant in our cohort. One patient had an association of granulomatous disease, autoimmunity, lymphadenopathy, and splenomegaly in the context of respiratory infection.

Table 1. Demographic and epidemiological features of 20 Moroccan CVID patients

Characteristics	Clinical data
Sex	10 females, 10 males
Consanguinity %	60%
Year of birth (± SD)	26.10 (± 14.23)
Age at onset, y (± SD)	13.85 (± 12.9)
Age at diagnosis, y (± SD)	18.50 (± 13.8)
Splenomegaly	50%
Lymphadenopathy	40%
Granulomatous disease	25%
Autoimmune phenomena	45%
Respiratory infection	90%
Vaccine response	Impaired (9 patients)

CVID: Common variable immunodeficiency, SD: Standard deviation

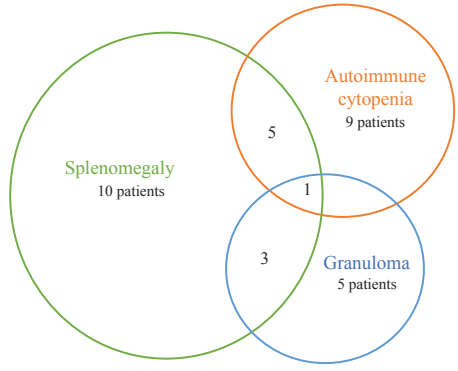


Figure 1. Coincidence of non-infectious complications in Moroccan CVID patients.
The cooccurrence of autoimmune cytopenia, granulomatous manifestations, and splenomegaly in 20 Moroccan CVID patients is illustrated. A significant correlation is observed between splenomegaly and autoimmunity, and to a lesser extent between splenomegaly granulomatous disease. The association of autoimmunity with granulomatous manifestations was not significant. *CVID: Common variable immunodeficiency*

Immunophenotyping with Flow Cytometry

To study the antigen-independent and -dependent differentiation of B-cells, six subpopulations of B-cells were distinguished as, naive B-cells ($IgM^+IgD^+CD27^-$),

transitional B-cells ($CD38^{hi}IgM^{hi}$), marginal zone-like B cells ($IgM^{+}IgD^{+}CD27^{+}$), activated B-cells ($CD21^{low}CD38^{low}$), switched memory B-cells ($IgD^{-}CD27^{+}$), and class-switched plasmablasts ($CD38^{+++}IgM^{-}$). B-cell subsets of CVID patients (n=17) and age-matched healthy controls (n=30) were analyzed using flow cytometry (Figure 2). After comparing the percentage of B-cell subpopulations in patients with controls, we noted a significant reduction in total ($CD19^{+}$) B-cells in patients (9.76 ± 6.81%) compared to healthy donors (75.3 ± 4.14%; p<0.001). We also found a significant reduction in switched memory (1.12 ± 2.67% vs. 13.55 ± 3.66%; p<0.001), marginal zone-like (1.57 ± 2.43% vs. 11.32 ± 3.73%; p<0.001), and transitional B-cells (1.10 ± 3.65% vs. 2.83 ± 1.6%; p<0.83), in addition to decreased class-switched plasmablasts (0.04 ± 0.09% vs. 1.71 ± 1.2%; p<0.001), in patients compared to controls. In contrast, the percentage of activated B-cells was significantly increased in patients (16.65 ± 27.25%) compared to controls (2.28 ± 0.97%; p<0.001). Naive B-cells were also relatively, but not significantly, higher in patients (83.69 ± 29.02%) compared to controls (79.9 ± 12.84%; p=0.45).

Classification of Moroccan CVID Patients

According to the European classification of CVID patients, we divided our cohort of patients into two main groups, those with B-cells count equal to or under 1% (group B-, 3 patients) and those with B cells count over 1% (group B^{+}, 17 patients) (Figure 3). Patients in group B^{+} were subclassified into two groups based on switched memory B-cells, group smB- with ≤2% switched memory B-cells (smB-, 15 patients), and group smB^{+} having >2% switched memory B-cells (2 patients). The occurrence of noninfectious complications of CVID was higher in group smB^{-}, compared to group smB^{+}, particularly splenomegaly (8/9 patients in smB^{-} vs 1/9 patients in smB^{+}), granulomatous disease (4/4 patients in smB^{-} vs. no patients in smB^{+}) and autoimmune manifestations (6/8 patients in smB^{-} vs. no patients in smB^{+}).

The smB^{-} group was further divided into two subgroups based on the number of transitional B-cells. The subgroup smB^{-} Tr^{hi} (2 patients), with ≥9% of transitional B-cells, showed more incidence of splenomegaly (1/2 patients, 50%), lymphadenopathy (1/2 patients, 50%), and granulomatous disease (2/2 patients, 100%) compared to the subgroup of smB^{-} Tr^{norm} (13 patients), with lesser than 9% of transitional B-cells, which shows respective incidence rates of splenomegaly, lymphadenopathy, and granulomatous disease in 47% (7/13 patients), 33% (5/13 patients), and 15% (2/13 patients) of patients (Figure 3).

Furthermore, for both groups smB^{+} and smB^{-}, subgroups with normal or expansion of $CD21^{low}$ B-cells above 10% of total B-cells are labeled (21^{norm} or 21^{low}). The expansion of $CD21^{low}$ B-cells was much more prevalent in the group without switched memory B-cells smB^{-} (11/15 patients) compared to group smB^{+} (1/2 patients). In the smB^{+} group (2/2 patients), one patient with an expansion of $CD21^{low}$ B-cells had an incidence of splenomegaly and autoimmune cytopenia, while the other patient in the smB^{+} CD21norm group had only autoimmune manifestations. As for the smB^{-} group, the incidence of splenomegaly (4/4 patients)

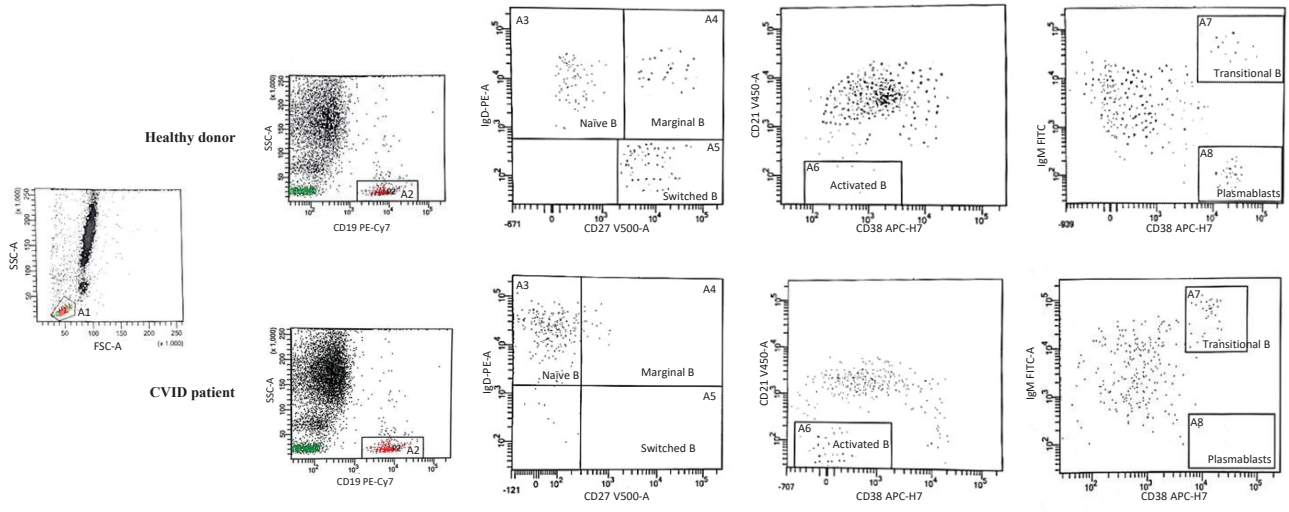


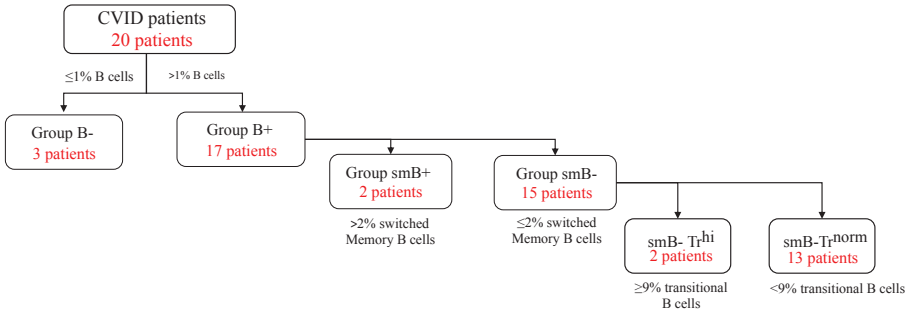
Figure 2. Flow cytometric dissection of B-cell subpopulations in CVID patient and age-matched healthy donor.
Flow cytometric evaluation of peripheral blood samples from one CVID patient and age-matched healthy donor is represented. B-cells were characterized by CD19 gating (A2) within the lymphocyte scatter area (A1). Naïve B-cells (A3), marginal zone B-cells (A4), and switched memory B-cells (A5) can be recognized by staining for CD27 and IgD. Activated B-cells ($CD38^{low}$ $CD21^{low}$) (A6), transitional B-cells ($CD38^{high}$ IgM^{high}) (A7), and plasmablasts ($CD38^{high}IgM^{low}$) (A8) can be identified by staining for CD21 and CD38. *CVID: Common variable immunodeficiency*

and autoimmunity (2/4 patients) was higher in smB$^-$ CD21^{norm} patients than in smB$^-$ CD21^{low} patients (5/11 and 4/11 patients, respectively). The incidence ($p<0.05$) of lymphadenopathies and granulomatous diseases was higher in smB$^-$ CD21^{low} patients (5/11 and 4/11) compared to patients in the smB$^-$ CD21^{norm} subgroup (Figure 4).

Other immunological investigations in CVID patients included total CD3$^+$ T-cells, CD4$^+$, and CD8$^+$ T-cells, as well as natural killer (NK) cells (CD16$^+$CD56$^+$). Seventeen patients were found to have normal T-cell counts (737-3.663/mm^3, reference 486-3.469/mm^3), total CD4 (302-1.827/mm^3, reference 251-2.180/mm^3), and CD8 T-cell (163 -2.385/L, reference 185-820/mm^3) and NK-cell numbers (14-575/mm^3, reference range 45-546/mm^3; data not shown).

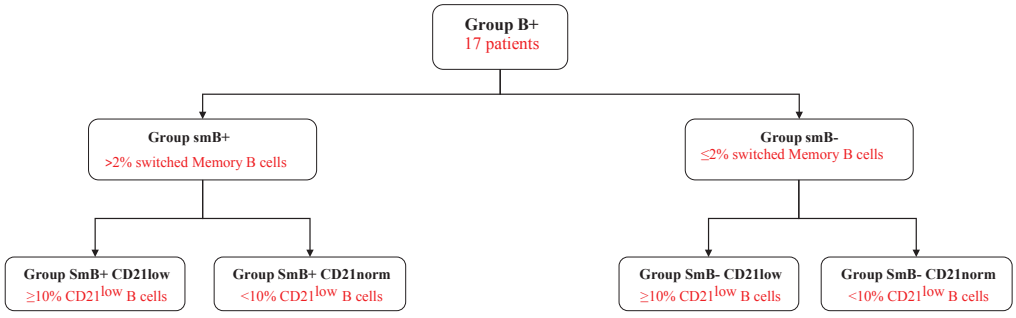
Discussion

CVID is a group of inborn errors of immunity that are clinically, immunologically, and genetically heterogeneous (12).



	Group B-	Group B+	smB+	smB-	smB- Tr^{hi}	smB-Tr^{norm}
Splenomegaly (n=9)	0/3	9/17	1/9 (10%)	8/9 (90%)	1/2 (50%)	7/13 (54%)
Lymphadenopathy (n=6)	2/3	6/17	0/6 (0%)	6/6 (100%)	1/2 (50%)	5/13 (38%)
Granuloma (n=9)	1/3	4/17	0/4 (0%)	4/4 (100%)	2/2 (100%)	2/13 (15%)
Autoimmunity (n=8)	1/3	8/17	2/8 (25%)	6/8 (75%)	0/2 (0%)	6/13 (46%)

Figure 3. Classification of 20 Moroccan CVID patients.
CVID: Common variable immunodeficiency



1	1	Number of patients	11	4
1/1 (100%)	0/1 (0%)	Splenomegaly	5/11 (45%)	4/4 (100%)
0/1 (0%)	0/1 (0%)	Lymphadenopathy	5/11 (45%)	1/4 (25%)
0/1 (0%)	0/1 (0%)	Granuloma	4/11 (36%)	1/4 (25%)
1/1 (100%)	1/1 (100%)	Autoimmunity	4/11 (36%)	2/4 (50%)

Figure 4. Classification of 17 Moroccan CVID patients in Group B$^+$.
CVID: Common variable immunodeficiency

Patients have in common a clear, even if incomplete, antibody deficiency, resulting in recurrent infections, mainly bacterial, and often respiratory (14). Recurrent and severe lung infections may lead to bronchiectasis and eventually chronic respiratory impairment (15). Ig replacement therapy helps to counter the gravity and reduce the incidence of these infections, yet they still pose a threat to CVID patients (16). There may also be gastrointestinal bacterial, and less frequently viral and/or parasitic, infections responsible for malabsorption, especially when there is an associated IgA deficiency. These infections manifest in form of chronic or acute diarrhea and Ig replacement therapy does not provide consistent improvement in these symptoms (17). In addition, other non-infectious manifestations, particularly autoimmunity, granulomatous diseases, and malignancy may also develop as complications. These manifestations of immune dysregulations are not ameliorated by Ig therapy (18).

The pathogenesis of CVID involves various genetic, epigenetic, and immunological defects (12). The immunological defects include abnormalities in the innate and adaptive immunity, in particular abnormal number and/or function of B and T lymphocytes (19,20). In most CVID cases, B-cell abnormalities are referred to defects in the late stages of B-cell differentiation (20). Approximately 90% of CVID patients were shown to have normal total B-cell counts but disturbed B-cell subsets (19,20). B-cell development undergoes successive stages of maturation beginning in the bone marrow and ending in the peripheral lymphoid organs. During this, hematopoietic stem cells go through several stages of differentiation, including the pro-B and pre-B-cell stages, before they become immature B-cells (20). Immature B-cells go through two transitional B-cell phases before becoming either marginal zone or naive follicular B-cells. Marginal zone B-cells evolve into IgM memory B-cells, while follicular B-cells differentiate into switched memory B-cells and plasma cells in germinal centers (20). Many reports have declared reduced IgM memory B-cells, class-switched memory B-cells, and plasma cells in CVID patients (8,9,21). This decrease may also result from increased apoptosis in the later stages of B-cell development (22,23). In addition, defective antibody production in the context of normal total B-cell counts could reflect an abnormality in the germinal center response, in which B-cells develop into memory and plasma B-cells (19).

The broad phenotype of CVID, as well as its heterogeneous clinical and immunological features, lead to distraction and delay in diagnosis, which in turn exacerbate related complications. The EUROclass represents a key instrument for early prediction, diagnosis, and treatment of non-infectious complications of CVID (10,11). This classification relies on the percentage of circulating B-cell subsets to classify patients into groups, notably patients with ≤2% switched memory B cells (group smB^-), ≥9% transitional B-cells (group smB^- Tr^{hi}), and/or with the expansion of $CD21^{low}$ B-cells to ≥10% (group $CD21^{low}$). In accordance with this classification, 88% of our patients showed reduced numbers of switched memory B-cells (group smB^-), including all patients with granulomatous affections and 90% of patients with splenomegaly in the cohort. It was shown that markedly decreased switched memory B-cells was linked with increased risk for splenomegaly and granulomatous affections (10). Those patients have lower IgA and IgG serum levels than those in group smB^+. Thus, this condition may be attributed to a disturbed germinal center function, where B-cells differentiate into switched memory B-cells and effector plasma B-cells (10,11). Patients with a high expansion of transitional B-cells (group smB^- Tr^{hi}) have a significant occurrence of lymphadenopathy, granulomatous affections, and splenomegaly without autoimmunity, compared to smB^- Tr^{norm} patients with a higher incidence of autoimmunity. smB^- Tr^{hi} patients have significantly reduced marginal zone-like B-cells compared to other patients, suggesting a complex immune dysregulation in these patients.

Moreover, the expansion of $CD21^{low}$ B-cells is strongly associated with splenomegaly, and this association is independent of the reduction of switched memory B-cells (10). In our cohort, the incidence of splenomegaly was higher in $CD21^{low}$ patients, notably in the smB^+ group, compared to patients with a normal expansion of $CD21^{low}$ B cells, similar to previous reports (10). Regarding the smB^- group, a slightly higher incidence of splenomegaly and autoimmunity was noted in $CD21^{norm}$ patients compared to $CD21^{low}$ patients, in contrast to what has been previously reported (10). This may be related to the late onset and the high delay in diagnosis observed in the group $CD21^{norm}$ compared to group $CD21^{low}$ (11), and the small number of CVID-diagnosed Moroccan patients in this cohort. It also remains uncertain if marginal zone-like B-cells decline along with the expansion of $CD21^{low}$ B-cells preceding or following splenomegaly (11). Besides, in about 10% of patients with CVID (20) and 15% of patients in our cohort, circulating B-cells represent less than 1% of lymphocytes (group B^-). Those patients suffer from the early onset of respiratory infections (median age of onset 5 years) attributed to an early differentiation defect of the B-cell lineage. Those patients were not enrolled for further analysis in this research. However, future studies should reexamine this group of CVID patients.

Conclusion

We reported the first evaluation and classification of a Moroccan cohort of CVID patients using the measurement

of B-cell subsets. The subgroups formed based on the EUROclass showed agreement in the noted correlation between clinical and immunological features in this study with results from previous studies, which confirmed the reliability of this classification and indicates its applicability in other populations. This will help to establish a globally applicable classification and diagnostic criteria with patients registered according to them in international registries. This, in turn, will help optimize the outcome of the approach. While these studies have helped to elucidate the role of B cell subsets in the pathogenesis of CVID and its complications, the description of new monogenic defects among CVID patients will increase our understanding of the molecular mechanisms behind this complex defect. This might open the way for new targeted therapeutic options other than immunoglobulin replacement therapy.

Ethics

Ethics Committee Approval: This study received ethical committee clearance (Centre Hospitalier Universitaire Ibn Rochd; approval number: 220/DOEHRS1/013).

Informed Consent: Informed consent from all patients were obtained.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: F.A., A.A.B., M.M., Concept: K.M., A.A., F.A., J.E.B., K.O., E.A., A.A.B., M.M., Design: K.M., J.E.B., A.A.B., Data Collection or Processing: K.M., A.A., Analysis or Interpretation: K.M., A.A., J.E.B., K.O., A.E., Literature Search: K.M., A.A., A.E., Writing: K.M., A.A., A.E.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declare that they have no relevant financial.

References

1. Aghamohammadi A, Farhoudi A, Moin M, Rezaei N, Kouhi A, Pourpak Z, et al. Clinical and immunological features of 65 Iranian patients with common variable immunodeficiency. Clin Diagn Lab Immunol. 2005:12:825-32.
2. Aghamohammadi A, Abolhassani H, Moazzami K, Parvaneh N, Rezaei N. Correlation between common variable immunodeficiency clinical phenotypes and parental consanguinity in children and adults. J Investig Allergol Clin Immunol. 2010;20:372-9.
3. Bousfiha A, Moundir A, Tangye SG, Picard C, Jeddane L, Al-Herz W, et al. The 2022 Update of IUIS Phenotypical Classification for Human Inborn Errors of Immunity. J Clin Immunol. 2022;42:1508-20.
4. Hammarstrom L, Vorechovsky I, Webster D. Selective IgA deficiency (SIgAD) and common variable immunodeficiency (CVID). Clin Exp Immunol. 2000;120:225-31.
5. Modell V, Knaus M, Modell F, Roifman C, Orange J, Notarangelo LD. Global overview of primary immunodeficiencies: a report from Jeffrey Modell Centers worldwide focused on diagnosis, treatment, and discovery. Immunol Res. 2014;60:132-44.
6. Modell V, Orange JS, Quinn J, Modell F. Global report on primary immunodeficiencies: 2018 update from the Jeffrey Modell Centers Network on disease classification, regional trends, treatment modalities, and physician reported outcomes. Immunol Res. 2018;66:367-80.
7. Shillitoe B, Bangs C, Guzman D, Gennery AR, Longhurst HJ, Slatter M, et al. The United Kingdom Primary Immune Deficiency (UKPID) registry 2012 to 2017. Clin Exp Immunol. 2018;192:284-91.
8. Warnatz K, Denz A, Drager R, Braun M, Groth C, Wolff-Vorbeck G, et al. Severe deficiency of switched memory B cells (CD27(+) IgM(-)IgD(-)) in subgroups of patients with common variable immunodeficiency: a new approach to classify a heterogeneous disease. Blood. 2002;99:1544-51.
9. Piqueras B, Lavenu-Bombled C, Galicier L, Bergeron-van der Cruyssen F, Mouthon L, Chevret S, et al. Common variable immunodeficiency patient classification based on impaired B cell memory differentiation correlates with clinical aspects. J Clin Immunol. 2003;23:385-400.
10. Wehr C, Kivioja T, Schmitt C, Ferry B, Witte T, Eren E, et al. The EUROclass trial: defining subgroups in common variable immunodeficiency. Blood. 2008;111:77-85.
11. Yazdani R, Seify R, Ganjalikhani-Hakemi M, Abolhassani H, Eskandari N, Golsaz-Shirazi F, et al. Comparison of various classifications for patients with common variable immunodeficiency (CVID) using measurement of B-cell subsets. Allergol Immunopathol (Madr). 2017;45:183-92.
12. Driessen GJ, van Zelm MC, van Hagen PM, Hartwig NG, Trip M, Warris A, et al. B-cell replication history and somatic hypermutation status identify distinct pathophysiologic backgrounds in common variable immunodeficiency. Blood. 2011;118:6814-23.
13. Bergman P, Wullimann D, Gao Y, Wahren Borgstrom E, Norlin AC, Lind Enoksson S, et al. Elevated CD21(low) B Cell Frequency Is a Marker of Poor Immunity to Pfizer-BioNTech BNT162b2 mRNA Vaccine Against SARS-CoV-2 in Patients with Common Variable Immunodeficiency. J Clin Immunol. 2022;42:716-27.
14. Conley ME, Notarangelo LD, Etzioni A. Diagnostic criteria for primary immunodeficiencies. Representing PAGID (Pan-American Group for Immunodeficiency) and ESID (European Society for Immunodeficiencies). Clin Immunol. 1999;93:190-7.
15. Verma N, Grimbacher B, Hurst JR. Lung disease in primary antibody deficiency. Lancet Respir Med. 2015;3:651-60.
16. Quinti I, Soresina A, Guerra A, Rondelli R, Spadaro G, Agostini C, et al. Effectiveness of immunoglobulin replacement therapy on clinical outcome in patients with primary antibody deficiencies: results from a multicenter prospective cohort study. J Clin Immunol. 2011;31:315-22.
17. Malamut G, Verkarre V, Suarez F, Viallard JF, Lascaux AS, Cosnes J, et al. The enteropathy associated with common variable immunodeficiency: the delineated frontiers with celiac disease. Am J Gastroenterol. 2010;105:2262-75.
18. Fischer A, Provot J, Jais JP, Alcais A, Mahlaoui N, members of the CFPIDsg. Autoimmune and inflammatory manifestations occur frequently in patients with primary immunodeficiencies. J Allergy Clin Immunol. 2017;140:1388-93:e1388.

19. Ahn S, Cunningham-Rundles C. Role of B cells in common variable immune deficiency. Expert Rev Clin Immunol. 2009;5:557-64.

20. Warnatz K, Schlesier M. Flowcytometric phenotyping of common variable immunodeficiency. Cytometry B Clin Cytom. 2008;74:261-71.

21. Carsetti R, Rosado MM, Donnanno S, Guazzi V, Soresina A, Meini A, et al. The loss of IgM memory B cells correlates with clinical disease in common variable immunodeficiency. J Allergy Clin Immunol. 2005;115:412-7.

22. Ganjalikhani-Hakemi M, Yazdani R, Esmaeili M, Abolhassani H, Rae W, Azizi G, et al. Role of Apoptosis in the Pathogenesis of Common Variable Immunodeficiency (CVID). Endocr Metab Immune Disord Drug Targets. 2017;17:332-40.

23. Yazdani R, Fatholahi M, Ganjalikhani-Hakemi M, Abolhassani H, Azizi G, Hamid KM, et al. Role of apoptosis in common variable immunodeficiency and selective immunoglobulin A deficiency. Mol Immunol. 2016;71:1-9.