Common Variable Immune Deficiency in Children—Clinical Characteristics Varies Depending on Defect in Peripheral B Cell Maturation

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Abstract Common variable immune deficiency (CVID) is a heterogeneous disease associated with ineffective production of antibodies. It is usually diagnosed in adulthood, but a variable proportion of children develop CVID. Early identification of patients with potentially worse prognosis may help to avoid serious complications. The goal of this study was to associate the clinical phenotype of patients with early-onset CVID with peripheral B-cell maturation profile. Four color flow cytometry was used to define distribution of peripheral B-cell subsets in 49 children with early-onset CVID. All clinical data were extracted from medical records. A proportion of patients demonstrated diminishing with time total B-lymphocytes pool, beyond physiological age-related changes. Irrespective from duration of the follow-up period the B-cell maturation profile in individual patients remained unchanged. We identified six different aberrant peripheral B cell maturation profiles associated with different clinical characteristics. Patients with an early B-cell maturation block earlier required replacement therapy and were at significantly greater risk of enteropathy, granuloma formation, cytopenia, and lymphoproliferation. B-cell maturation inhibited at the natural effector stage was associated with higher risk of autoimmune manifestations other than autoimmune cytopenia. Prevalence of male patients was observed among patients with B-cell maturation inhibited at naïve B-cell stage. In conclusion, the diagnostic process in patients with suspected early-onset CVID shall include routine analysis of peripheral B-cell maturation to provide surrogate markers identifying patients at greater risk of developing certain complications.

Keywords Common variable immune deficiency · flow cytometry · B lymphocytes · defective B-cell maturation

Introduction

Common variable immune deficiency (CVID) is a heterogeneous disease characterized by hypogammaglobulinemia, defective antibody responses and recurrent infections [1, 2]. It is associated with an increased susceptibility to autoimmune disorders and malignancies [3–6]. The characteristic immunologic defect is an ineffective differentiation of B-lymphocytes into memory cells [7, 8] and further into plasma cells capable of secreting all immunoglobulin types [9, 10]. CVID is usually diagnosed in second or third decade of life, but a variable proportion of children presenting with antibody deficiency...
(AD) develop CVID during the follow-up time [11–13]. The diagnosis in children is particularly difficult due to immunologic immaturity and the persistence of transient hypogammaglobulinemia of infancy in some children.

In attempt of identifying patients with potentially worse prognosis several classification schemes have been developed based on abnormalities in B cell phenotyping [14–16]. Enumeration of memory cells in CVID has been proposed as a prognostic marker of respiratory disease [8, 13, 15, 17], splenomegaly [8, 17], autoimmunity [8, 18], granuloma formation [8, 15, 16, 18], and intestinal involvement [8]. Loss of IgM-only memory B cell subset has been correlated with an increased risk of chronic respiratory infections potentially leading to bronchiectasis [19], while the expansion of CD21low population was associated with autoimmune cytopenia [20].

Scarce attempts to describe features of CVID specific for children point to differences between pediatric and adult patients. Autoimmune cytopenia as the first symptom of the disease [21], marked delay of diagnosis due to overlap with common pediatric disorders [22], a substantial prevalence of bronchiectasis [23], sensitivity to ionizing radiation [24], and prolonged observation required to establish the diagnosis, are among few of these differences [25].

Considering significant age-related changes in the distribution of cell subsets reflecting major B lymphocyte maturation stages [26–28] it is likely that current classification systems of CVID are not directly applicable to pediatric population. The goal of this study was to summarize long-term clinical observations of a well-defined population of pediatric patients who fulfilled criteria of the European Society for Immune Deficiencies (ESID) for probable diagnosis of CVID and to associate the clinical condition of patients with peripheral B cell maturation profile [2].

Material and Methods

The study group included 49 children (18 females and 31 males, median age 10.2 years (3.1–17.5 years)) referred to the Department of Clinical Immunology of the Children’s Memorial Health Institute (Warsaw, Poland) between September 1995 and September 2011 with diagnosis of probable CVID according to ESID criteria [2].

Clinical and laboratory data of patients were collected retrospectively from medical records. All children were older than 2 years at first clinical manifestations and fulfilled ESID criteria for diagnosis of probable CVID, i.e.: demonstrated significantly reduced serum IgG, IgA and/or IgM levels below age-matched normal values [29], poorly responded to vaccination, and/or had low isohemagglutinin titers. Other defined causes of hypogammaglobulinemia have been excluded.

Clinical Data

Documented clinical data, such as history of recurrent or chronic infections, lymphadenopathy, organomegaly, autoimmune cytopenias and other autoimmune phenomena, such as granuloma formation and enteropathy were included in a standardized questionnaire. Date of first symptoms associated with immune deficiency, date of first diagnosis of aberrant immunoglobulin levels, date of initiation of replacement therapy, as well as serum immunoglobulin levels before replacement therapy and any significant alteration of IgA or IgM levels thereafter were recorded.

One of the authors has seen and followed sequentially all patients enrolled in the study. X-linked agammaglobulinemia (XLA) was excluded in male patients with low B cell numbers by evaluation of Btk expression by flow cytometry or western blot (results not shown). Mutation analysis for any of the rare gene defects associated with CVID such as CD19, ICOS, BAFFR or TACI deficiency, was not performed.

B Cell Compartment Analysis

The B-cell compartment was analyzed by four color flow cytometry using whole EDTA-K2 anticoagulated blood and monoclonal antibodies (Table I) as described previously [30, 31]. Differential expression of CD19, IgD, IgM, CD21, CD27, and CD38 allowed to determine five subsets reflecting major B lymphocyte maturation stages: transitional CD19−CD38++IgM++, naïve CD19+IgD−CD27−, natural effectors CD19−IgD−CD27+, memory CD19−IgD−CD27+ B cells, and CD19+IgM+CD38++ plasmablasts (Fig. 1). A population of CD19+CD21lowCD38low B lymphocytes represented activated B cells [32]. All results were analyzed in context of age-matched normal values determined in the previously described control group of 132 healthy subjects (32 children aged 2–5 years, 30 children aged 5–10 years, 42 children aged 10–16 years, and 28 healthy young adults older than 16 years) [30]. Results below 5th or above 95th percentile of the age-matched normal range were considered abnormal. All samples were run using the same methodology and results were reviewed by the same person experienced in flow cytometry. B-cell maturation profile was determined more than once (2–6 times) in at least 3 months interval in 36 patients from the study cohort; in the remaining 13 patients only single determination was performed (Table II).

Statistical analysis was performed with Mann–Whitney U and Fisher exact tests.

The study was approved by an institutional review board and carried according to the guidelines of Helsinki Declaration. Written consent was obtained from parents and children, if older than 16 years.
Results

Clinical Phenotype

The clinical picture was highly variable. Recurrent respiratory tract infections were reported in 42 patients (85.7 %); among them 32 patients suffered from bronchitis and/or pneumonia, sinusitis was diagnosed in 21 patients, otitis media in 17, and pharyngitis in seven. Bronchiectasis was detected by high-resolution computer tomography (HRCT) in eight patients, while pulmonary fibrosis was confirmed by histopathology in five patients. Recurrent respiratory system infections as a first manifestation of the immune insufficiency were reported in 34 patients (69.4 %).

Splenomegaly was observed in 27 patients (55.1 %), hepatomegaly in 18 patients (36.7 %), while lymphadenopathy affected 35 patients (71.4 %), including one patient for whom persistent lymphadenopathy prompted the diagnostic process. Hepatosplenomegaly alone or accompanied by lymphadenopathy, or otitis, or autoimmune hemolytic anemia (AIHA) as first manifestation of the disease were observed in one patient each.

Cytopenias were reported in 17 patients (34.7 %), among them mainly thrombocytopenia (16 patients) (32.7 %), with isolated thrombocytopenia observed in seven patients (14.3 %), and thrombocytopenia accompanied by leukopenia or autoimmune anemia—in 8 (16.3 %) and four patients (8.2 %), respectively. One patient experienced thrombocytopenia, neutropenia, and anemia. Isolated thrombocytopenia as the first symptom of immune insufficiency was described in three, while autoimmune hemolytic anemia in two patients.

Other autoimmune manifestations affected six patients (12.2 %) and included arthritis (two patients), autoimmune

| Tube | FITC Specificity/Clone | PE Specificity/Clone | PerCP Specificity/Clone | APC Specificity/Clone |
|------|------------------------|----------------------|-------------------------|-----------------------|
| 1    | CD21/LB21              | IgD/IA6-2            | CD19/4G7                | CD27/L128             |
| 2    | IgM/G20-127            | CD21/LB21            | CD19/4G7                | CD38/HIT2             |

Fig. 1 Immunophenotyping of peripheral B lymphocytes based on differential expression of CD19, IgD, IgM, CD21, CD27, CD38. B lymphocytes were identified as cells with positive expression of CD19 and low side scatter (1). Recent bone marrow emigrants (transitional B cells) have been defined as B lymphocytes demonstrating high surface expression of IgM and CD38 (2). B lymphocytes with positive surface expression of IgD, but lacking expression of CD27 were identified as naïve B cells (3). Natural effectors expressed both IgD and CD27 (4), while memory B lymphocytes expressed CD27, but did not express IgD (5). Plasmablasts were identified as B lymphocytes with positive expression of CD38, but lacking expression of IgM (6). B lymphocytes with low expression of CD21 and CD38 were considered as activated B lymphocytes (7).
thyroiditis (two patients), and autoimmune hepatitis (two patients).

Granuloma formation was described in five patients (10.2%); among them one patient demonstrated granulomatous gingival hyperplasia as the first clinical manifestation of the immune defect. Granulomas in lungs were confirmed by histopathology in three patients, while presence of liver granulomas was confirmed by histopathology one patient.

Eleven patients (22.4%) demonstrated symptoms of enteropathy. In one patient celiac disease was diagnosed based on biopsy results and presence of anti-gliadin antibodies. Ulcerous colitis and inflammatory bowel disease were diagnosed based on biopsy results in one and two patients, respectively. The remaining seven patients suffered from recurrent diarrhea which resolved after initiation of regular immunoglobulin substitution therapy.

Humoral Immune Abnormalities

Although first clinical symptoms of immune insufficiency were reported since median age of 4 years (2.0–16.3 years), dysgammaglobulinemia was first detected at median age of 8.8 years (2.4–17.3 years). Median delay between first symptoms related to immune deficiency and diagnosis of dysgammaglobulinemia was 2.4 years (0–12.2 years). Selective IgA deficiency as the first defect was observed in four patients (8.2%) at median age of 3.2 years (0.8–15.4 years), while selectively reduced IgG levels were first observed in ten patients at median age of 7.2 years (4.0–17.0 years). In one patient transient hypogammaglobulinemia of infancy (THI) was diagnosed based on recurrent infections accompanied by reduced IgG levels in early childhood, that resolved spontaneously by the age of 2 years and 4 months.

Despite periodical elevation of serum IgM levels in six patients (12.2%) (one boy and five girls), all currently known gene defects causing hyper-IgM syndrome were excluded (results not shown).

Substitution therapy due to severe clinical condition accompanying hypogammaglobulinemia was initiated at median age of 11.0 years (min. 3.0, max 17.6 years), with the median interval between first observation of aberrant serum immunoglobulin levels and initiation of replacement therapy of 3.6 months (0–160 months).

Alterations in B Cell Compartment

A proportion of patients for whom the B-cell profile was determined at least three times in at least 1 year interval, demonstrated gradual reduction of total B lymphocyte counts. Despite lack of specific anti-B cell treatment with anti-CD20 monoclonal antibody in five patients total B-cell counts declined from normal to below 5th percentile of the normal age-matched range (see Fig. 2). Irrespective from the duration of the follow-up period and changes in total B-lymphocyte counts, the B-cell maturation profile in individual patients remained mostly unchanged (results not shown).

We identified six different aberrant B cell peripheral maturation profiles (Fig. 3 and Table III). Two patients with reduced total B lymphocyte counts, normal proportion of naïve CD19+IgD−CD27− B lymphocytes, and significantly reduced transitional, memory, and plasmablast subsets were assigned to group I. Immunophenotyping of B cell
Fig. 2 Despite lack of treatment with anti-CD-20 monoclonal antibody a proportion of patients demonstrated gradual decline in total B cell counts, beyond age-matched normal changes. Individual patients’ data are presented as dots on box-and-whisker plots of respective age groups, with the boxes representing the interquartile (25–75 percentiles) and the whiskers representing the 5–95 percentiles of the age-related normal range.

Fig. 3 Patients from the study cohort were assigned into six groups reflecting the identified B-cell maturation blocks. Patients with reduced total B cell counts with poor ability to mature beyond naïve stage, were included in group I. Group II was composed of patients who accumulated transitional B cells. Patients from group III accumulated naïve B-cells, while in patients from group IV B lymphocytes where unable to mature beyond natural effector B-cells. Patient from group V demonstrated normal B-cell maturation profile, except for reduced proportions of plasmablasts. Patient assigned to group VI demonstrated normal B-cell maturation profile. Individual patients’ data are presented as dots on box-and-whisker plots of respective age groups, with the boxes representing the interquartile (25–75 percentiles) and the whiskers representing the 5–95 percentiles of the age-related normal range.
Table III  Schematic representation of detected potential B-cell differentiation blocks in the study group in context of currently known B-cell maturation defects

| Putative developmental block | Bone marrow | Peripheral bloodcsl |
|-----------------------------|-------------|---------------------|
|                             | I           | II                  |
|                             | ↓           | ↓                   |
|                             | II          | III                 |
|                             | ↓           | ↓                   |
|                             | III         | IV                  |
|                             | ↓           | ↓                   |
|                             | IV          | V                   |
|                             | ↓           | ↓                   |
|                             | V           | VI                  |
|                             | ↓           | ↓                   |

| B cell subset Phenotype | Pre-B | Pre-BII | Immature | Transitional | Naive | Natural effector | Memory | Plasmablast |
|-------------------------|-------|---------|-----------|--------------|-------|------------------|--------|-------------|
| Pro-B                   | CD22+ | CD22+   | CD22+     | CD19+        | CD19+ | CD19+           | CD19+  | CD19+       |
| Pre-BI                  | CD34+ | CD34+   | CD34+     | IgM++        | IgD+  | IgD+            | IgD+   | IgM+        |
| CD19+                   | cTdT+  | cTdT+   | cTdT+     | cTdT+        | cTdT+ | cTdT+           | cTdT+  | cTdT+       |
|                          | sIgM+  | sIgM+   | sIgM+     | sIgM+        | sIgM+ | sIgM+           | sIgM+  | sIgM+       |

| Currently known defects |
|-------------------------|
| BTK                     |
| IGHM                    |
| BLNK                    |
| CD79A                   |
| CD79B                   |
| LI4.1                   |
| BAFFR                   |
| CD19                    |
| AID                     |
| CD81                    |
| UNG                     |
| CD21                    |
| PMS2                    |
| CD20                    |
| CD40                    |
| CD40L                   |
| ICOS                    |
| TACI                    |
transitional CD19+CD38++IgM++ B-cells, and significantly low relative numbers of B-lymphocytes, an accumulation of I to pre-B-II stage (results not shown).

Patients assigned to group II (n=9) demonstrated very low relative numbers of B-lymphocytes, an accumulation of transitional CD19+CD38++IgM++ B-cells, and significantly reduced proportions of more mature stages. Patients assigned to group III (n=24) demonstrated an accumulation of B-lymphocytes at naïve stage (CD19+IgD+CD27 -) of development and significantly reduced proportions of more mature stages (Fig. 2).

Patients assigned to group IV (n=12) accumulated natural effector B cells and demonstrated significantly reduced proportions of older maturation stages. One patient with normal distribution of all analyzed B-cell subsets, except for significantly reduced proportion of plasmablasts, was assigned to group V. One other patient with no apparent defect in B cell maturation, as defined by the analyzed B-cell subsets, was assigned to group VI (Fig. 2).

Due to low numbers of patients assigned to groups I, V and VI any comparison was possible only between patients from groups II, III, and IV (see Table II).

Variable Clinical Features Depending on B Cell Maturation Block

We observed several differences in clinical features between patient groups. Median age at first clinical presentation of the immune insufficiency and at initiation of replacement therapy was significantly lower in patients from group II than III (3.0 vs 5.6 years, p=0.0100547 and 7.4 vs 12.4, p=0.0057617, respectively). Other clinical features observed more frequently in patients assigned to groups II than III included enteropathy (55.6 % vs 12.5 %, p=0.0201), granuloma formation (33.3 % vs 0 %, p=0.0154), production of monoclonal or oligoclonal IgM (44.4 % vs 8.3 %, p=0.0342), as well as combined features of cytopenia and lymphoproliferation or cytopenia and enteropathy (both 44.4 % vs 4.2 %, p=0.0133). Although differences in proportions of patients demonstrating autoimmune cytopenia among the identified subgroups did not reach statistical difference, other autoimmune manifestations were more frequent among patients from group IV than III (Table II). Significantly higher proportion of male patients was observed among patients from group III (M:F=18:6), but not in other groups.

Discussion

Common variable immune deficiency is a complex, heterogeneous disease, with a common feature of ineffective production of high affinity antibodies [33]. The variability in time of the disease onset and clinical symptoms reflects the heterogeneity of defective mechanisms leading to abnormalities in B-cell survival [34], number of circulating CD27+ memory B lymphocytes [7, 8, 13–15, 17, 35], B cell activation after antigen receptor cross-linking [36, 37], T cell signaling [38], and cytokine expression [3, 39]. The genetic defect has been discovered for less than 20 % of patients [40].

The diagnostic criteria developed by the ESID first published in 1999 [2], were changing with time to exclude patients with other primary or secondary immune defects. According to currently valid criteria, none of the patients from the study cohort were able to mount T-cell dependent or T-cell independent antibody responses, as measured by post-vaccination response and low or lacking isohemagglutinins (if applicable), respectively [41, 42]. All patients were older than 4 years at the time of diagnosis, all criteria of probable CVID were met, and no other cause of hypogammaglobulinemia was found.

The outcome of CVID depends on interplay of several factors including sex, number of memory B lymphocytes and baseline immunoglobulin levels [43]. The prevalence of boys in the study group is consistent with the observation that male patients are generally more severely affected [3, 43], but it is not clear why this prevalence was observed only among patients demonstrating block at naïve stage of the B cell maturation process (group III) (Table II). It is tempting to speculate that a proportion of these patients may suffer from an unidentified yet, X-linked form of primary immune deficiency.

An increased susceptibility to recurrent respiratory infections, significant delay between first clinical symptoms and hypogammaglobulinemia are common in CVID in all age groups [1, 3, 11, 12, 17–19, 22, 44–52]. Severe complications in form of bronchiectasis and pulmonary fibrosis, observed in a minority of the study cohort, may be probably attributed to less cumulative respiratory tract infections than usually experienced by adult patients [8, 11, 12, 52–56].

The mechanism of granuloma formation in CVID patients is poorly understood [57]. Granulomatous lesions, demonstrated by 10.2 % of the study cohort, including three patients with features of granulomatous-lymphocytic interstitial lung disease (GLILD) associated with decreased patient survival [58], were significantly more frequent among patients with early B cell maturation defect (group II). Similar phenotype accompanied by an expansion of B cells at pre-naïve level has been observed in patients with chronic granulomatous disease [59].

Patients affected with CVID have an increased ability to produce antibodies against self-antigens, despite inability to produce appropriate levels of antibodies to bacterial or viral antigens [1, 3, 6, 12, 14–16, 22, 53, 60–62]. Autoimmune phenomena have been observed in as much as 42.9 % of the study cohort and frequently preceded other manifestations.
of the disease. In a notable proportion of patients, autoimmune cytopenias, especially autoimmune thrombocytopenia (AIT), were observed as the first manifestation of the disease. The relationship between the defect in B cell maturation process and AIT is not clear [61], but similar defects in asplenic patients may indicate that spleen of CVID patients provides an inadequate environment for efficient control of the platelet population [63].

Gastrointestinal tract is the second most frequently affected system in patients with CVID [3]. Enteropathy and autoimmunity were significantly more frequently observed among our patients with low B cell counts, maturation process inhibited at transitional stage (group II), and an expanded CD21low B cell subset possibly reflecting the activation status, than in other subgroups of the study cohort [13, 64, 65].

CVID may result in a panoply of other non-infectious complications, such as persistent lymphadenopathy [1, 14–16, 26] and splenomegaly [14–16, 26]. In contrast to previous reports, no direct association between splenomegaly and significant reduction of memory B lymphocytes or expanded CD21low population of B lymphocytes was identified [14–16]. None of the described potential B cell differentiation block sites in the investigated cohort could be associated with either of the manifestations. It is therefore possible that both clinical phenomena result from an increased frequency of infections.

B cell differentiation is a stepwise process involving several checkpoints. Patients from groups I and II, with low to extremely low peripheral B lymphocytes, combine features of patients with early B cell differentiation block similar to seen in Btk deficiency, described by Ochtrop [66]. At least one of our patients who fit into this description was however female and therefore rather unlikely to suffer from Btk deficiency, especially that her peripheral B cell counts repeatedly composed 6 % to 8 % of peripheral blood lymphocyte (PBL) pool [67].

An increased frequency of autoimmune phenomena among patients from group II was associated with an expanded population of CD21low cells, found to be preactivated, polyclonal, partially autoreactive B lymphocytes homing to peripheral tissues [32] and a subset of B lymphocytes lacking surface expression of IgD and CD27, potentially including CD27+ IgG+ cells with suggested role in autoimmunity [68]. The expanded subset of IgD-CD27- cells observed in this subgroup of patients, may possibly also contain IgA+ memory B cells [68] with potential role in normal production of serum IgA. The accumulation of naïve B lymphocytes observed in patients from group II may result from an increased proliferation compensating for a decreased bone marrow output [69, 70].

An impaired development of B-lymphocytes with natural effector phenotype in patients from group III precludes generation of plasma cells producing an efficient humoral response against encapsulated bacteria. Clinically this results in an increased susceptibility to respiratory infections. The B-cell subset composition in patients from this group may indicate for possible defects in B-cell receptor structure or function, including mutations in genes coding CD19 [71, 72], CD20 [73], or CD81 [74]. Patients share also some features of an inducible costimulator (ICOS) deficiency, which may present both in adults and children [75].

Defective generation of memory B cells not affecting other cell subsets, observed in patients from group IV, suggests a germinal center defect with normal proliferation, manifested by an increased number of natural effector B cells [69]. Due to extremely complex nature of the germinal center reaction (rev. by [76]), the clinical phenotype may result from several defects, including mutations in genes responsible for effective cooperation of T and B lymphocytes in germinal centers, such as in transmembrane activator and calcium-modulator and cyclophilin ligand interactor (TACI) [77]. This group probably encompasses also several other defects not associated with unique aberrancy in B lymphocyte pattern other than lack of memory B lymphocytes.

Reduced number of plasmablasts in a patient from group V and ineffective production of post-vaccination response demonstrated by the patient assigned to group VI, despite lack of an apparent block in peripheral B cell maturation, might reflect extrafollicular, follicular or terminal post-GC maturation defect, leading to an ineffective terminal plasma differentiation and poor generation of long-living plasma cells [69]. Genetic defects leading to such phenotype have not been identified yet. Potential candidates include defective Blimp-1, known to affect the generation of long-living plasma cells [9] or B-cell maturation antigen (BCMA), known to affect the generation of short-living plasma cells and serum levels of the immunoglobulins produced by these cells [78]. Studies on significantly greater populations of patients are necessary to find the mechanism of these defects.

Common variable immune deficiency is generally associated with severe reduction of post-germinal cells, with pre-germinal cells preserved in most patients [14]. Currently valid classification systems are based on differences in distribution in peripheral B-cell subsets reflecting maturation profiles, but referring to clearly defined cut-off values in proportions of respective cell subsets [14–16]. A striking observation in the long-term follow-up of a proportion of patients from the study cohort was significant decline with time in total B lymphocyte counts, despite lack of anti-B-cell targeted treatment. However, even in these patients the aberrancies in B cell profile remained stable, as reported by Kalina [79]. In an attempt of finding surrogate markers
identifying clinical phenotypes at higher risk of severe complications, we subdivided the study group depending on aberrant B cell maturation profile. We identified a group of patients, characterized by an early B cell maturation block, with significantly earlier manifestation of the disease, earlier need for replacement therapy, and significantly greater risk of enteropathy, granuloma formation, cytopenia and lymphoproliferation. We also identified a subgroup of patients with maturation profile inhibited at the natural effector/marginal zone-like stage at higher risk of autoimmune manifestations other than autoimmune cytopenia. No other significant association between B cell immunophenotype and clinical features were found in the analyzed study cohort.

In summary, results of this study show that it is an oversimplification that pediatric patients with few or absent memory B lymphocytes exhibit a different clinical phenotype than patients with higher numbers of memory B lymphocytes [13]. Results of this study present a description of the disease evolution, including evidence that a proportion of patients may also demonstrate diminishing with time total B-lymphocyte pool, beyond physiological age-related changes. The diagnostic process in recurrent manifestations of an unexplained origin in children older than 4 years, especially cytopenia, autoimmune or inflammatory process of unknown origin, shall therefore include routine periodical measurement of serum immunoglobulins and analysis of B cell phenotype to prevent incorrect treatment and development of further complications.

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