

# Pediatric common variable immunodeficiency: Immunologic and phenotypic associations with switched memory B cells

Yong PL, Orange JS, Sullivan KE. Pediatric common variable immunodeficiency: Immunologic and phenotypic associations with switched memory B cells.

*Pediatr Allergy Immunol* 2010.

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Recent studies suggest that patients with common variable immunodeficiency (CVID) and low numbers of switched memory B cells have lower IgG levels and higher rates of autoimmune disease, splenomegaly, and granulomatous disease; however, no prior literature has focused exclusively on pediatric cases. We examined the relationship between switched memory B cells and clinical and immunologic manifestations of CVID in a pediatric population. Forty-five patients were evaluated. Patients were categorized as Group I (<5 switched memory B cells/mL,  $n = 24$ ) or Group II ( $\geq 5$  switched memory B cells/mL,  $n = 21$ ).  $CD3^+$  T-cell counts and  $CD19^+$  B-cell levels were lower among Group I patients. Only those in Group I had meningitis, sepsis, bronchiectasis, granulomatous lung disease, autoimmune cytopenias, or hematologic malignancies. Segregation of pediatric patients into high risk (Group I) and average risk (Group II) may assist in targeting surveillance appropriately.

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Key words: common variable immunodeficiency; memory B cells; autoimmune disease; recurrent infections; IgG

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Accepted 26 January 2010

Common variable immunodeficiency (CVID), a disorder characterized by recurrent sinopulmonary infections, results from impaired antibody synthesis (1–5). In addition to infections, CVID can also result in a panoply of non-infectious complications. Significant pulmonary disease such as bronchiectasis and granulomatous lung disease can occur (2, 6–11). Other common manifestations of CVID include autoimmune and lymphoproliferative diseases, including hemolytic anemia and lymphoma, respectively (1, 5, 7, 12, 13). CVID can also affect the gastrointestinal tract, causing autoimmune hepatitis, a sprue-like syndrome, and intestinal lymphoid hyperplasia (6, 14). While CVID most

commonly manifests in the third decade of life, it can occur in children as young as 24 months of age (15). Studies have suggested that children with CVID may have a different phenotype than adults with CVID, with high rates of asthma, autoimmune disease, and growth hormone deficiency (16–19); however, these studies did not evaluate B-cell subsets. In general, diminished B-cell numbers and diminished switched memory B cell specifically are associated with a poorer outcome in CVID (7, 8, 20–28). While a few studies on switched memory B cells have included pediatric cases (7, 24, 29, 30), there are few reports defining clinical associations with diminished B cell in pediatric CVID.

The pathogenesis of CVID is complex, multiple defects in the developmental pathway from B cells to plasma cells have been associated with CVID, including mutations in the transmembrane activator and calcium-modulator and cytophilin ligand interactor (TACI), (31–33) and CD19 (34, 35). There may also be defects in the innate immune system as well (36). Patients with CVID have wide variation in B-cell function and the composition of their B-cell compartment.

As part of efforts to identify those patients at risk of significant complications and poorer prognoses, recent attention has focused on the relationship of memory B cells, as defined by the presence of cell-surface CD27, with clinical phenotype (20, 22, 23, 27). Memory B cells are classified as non-class-switched (producing IgM) and class-switched (producing IgG and IgA). Low numbers of class-switched memory B cells have been associated with lower levels of serum IgG, autoimmune disease, splenomegaly, and granulomatous disease in adult patients with CVID (7, 22–24, 26–28, 32, 33, 37). Stratification of adult patients with CVID on the basis of their B-cell phenotype has recently led to the development of a consensus document for adults (23) and standardization of flow cytometric methods for identifying B-cell subsets (38, 39). As children have higher and less mature B cells in their peripheral blood, the classification criteria cannot be directly applied to children, but the lesson of standardizing the approach to stratification is a valuable one, and our study contributes to that by providing important clinical data. Efforts are already underway to establish normative data in children and to define disease associations (24, 30), and recent studies have also identified a subset of children with CVID who have very low switched memory B cells (24, 29, 30). These studies did not evaluate phenotype. One study identified clinical phenotypes and suggested a correlation of low switched memory B cells with more severe disease but was not sufficiently powered to define clinical associations (8). Therefore, this study was undertaken to examine the relationship between switched memory B cell counts and clinical and immunologic manifestations of CVID in the pediatric population.

## Methods

All patients seen at The Children's Hospital of Philadelphia who met standard diagnostic criteria for CVID (40) and had a full evaluation of their peripheral blood lymphocytes (PBLs) were included in the study. All patients were seen by

one of the authors and followed sequentially with serial examinations by the same immunologist. CVID criteria included at least a two standard deviation decrease below the mean for age in two of the three major immunoglobulin isotypes (IgG and IgA or IgM); onset of the immunodeficiency at greater than 2 yr of age; absent isohemagglutinins and/or poor responses to vaccines; and exclusion of other defined causes of hypogammaglobulinemia (41–43). These correspond to the ESID and PAGID criteria (<http://www.esid.org>, <http://www.clinimmsoc.org>). Levels of serum immunoglobulins were measured prior to initiation of treatment using nephelometry. Poor responses to vaccines were confirmed by assessment after booster vaccines. X-Linked Agammaglobulinemia (XLA) was excluded as a diagnosis in males with low B cells by flow cytometry for Btk. Mutation testing was not performed for any of the rare gene defects associated with CVID such as CD19, ICOS, or BAFFR deficiency (34, 35, 44–47). TACI sequencing was not routinely performed and is not reported (48, 49).

Assessment of PBLs included enumeration of the CD3<sup>+</sup>4<sup>+</sup> T cell, CD3<sup>+</sup>8<sup>+</sup> T cell, CD19<sup>+</sup> B cell, CD20<sup>+</sup>, CD19<sup>+</sup>27<sup>+</sup>IgM<sup>+</sup>, and CD19<sup>+</sup>27<sup>−</sup>IgM<sup>−</sup> switched memory B-cell compartments by standard flow cytometry with labeled monoclonal antibodies in a CLIA-approved laboratory. Examples of patients' flow data are shown in Fig. 1. All samples were run in the same laboratory and reviewed by the same pathologist. In all but two patients, the B-cell findings were confirmed with at least two separate evaluations.

Abstractors utilized a standardized chart abstraction tool developed by the United States Immunodeficiency Network (<http://www.usid-net.org>), a research consortium established to advance research in primary immunodeficiencies by creating a registry of patients with primary immunodeficiency diseases. Over 500 data fields are included in the forms. Relevant clinical information from the patients' electronic medical records, which included inpatient and outpatient records as well as laboratory and pathology results, was extracted. A trained abstractor from USIDNET was used. Clinical conditions were reported only when confirmed by a relevant subspecialist. Patient observation periods ranged from 1–15 yr. Data were entered into a database and analyzed using STATA SE, version 10.0 (College Station, TX, USA). As the distribution of switched memory B cells was bimodal, we divided the cohort into two groups: Group I (<5 switched memory B cells/ml) and Group II (≥5 switched memory B cells/ml) (Fig. 2). Descriptive statistics were calculated, and

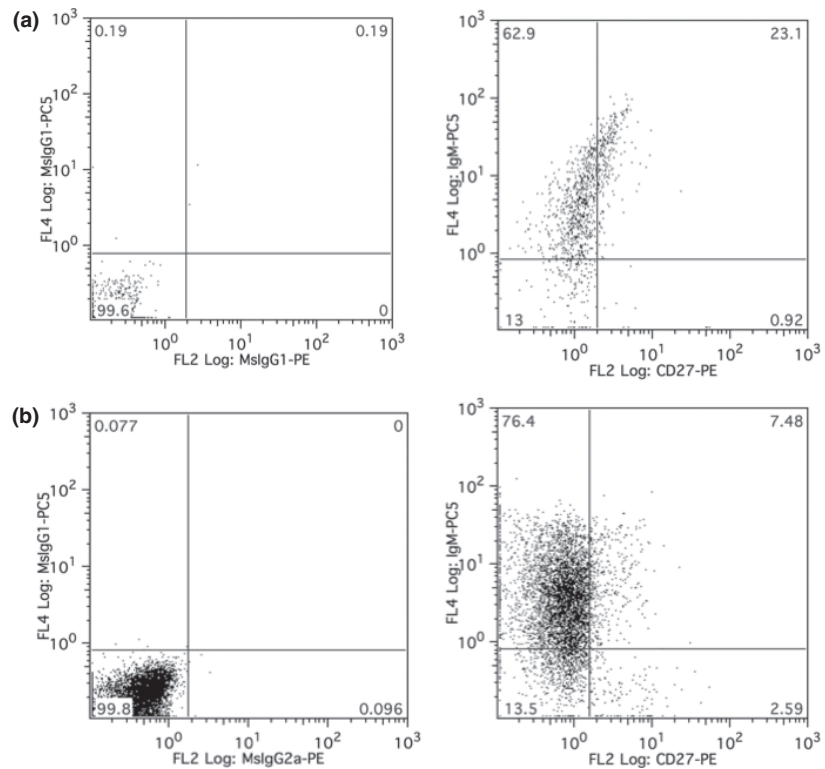


**Fig. 1.** Flow cytometry for switched memory B cells ( $CD19^+27^+IgM^-$ ). The cells were gated on CD45 and side scatter followed by CD19. The first panels represent the isotype controls. The second panels represent CD27 staining on the X-axis and IgM staining on the Y-axis. (a) A patient with minimal switched memory B cells (0.92%) also has a maturation that is disarrayed with increased non-switched memory B cells ( $CD19^+27^+IgM^+$ ). (b) A patient with normal B cell subsets, as defined by this staining strategy, is shown for comparison.

differences between the two groups were assessed using the Mann–Whitney test for continuous outcomes and Fischer’s exact test for categorical outcomes. The study protocol was approved by the CHOP Internal Review Board, and informed consent obtained for all patients.

## Results

### Laboratory features

The goal of this study was to determine whether the presence of low switched memory B cells in pediatric patients with CVID had the same clinical correlates as in an adult population. B-cell numbers are higher in children than in adults, and therefore we first surveyed the switched memory B-cell counts in our population (Fig. 2). It may be seen that the B-cell population is bimodal, and we therefore selected a cutoff of 5 cells/ml to divide our CVID population into Group I (low switched memory B cells) and Group II. Of the 45 patients included in this study, 24 (53%) patients were classified as Group I (Table 1). Group I ( $n = 24$ ) patients had a median switched memory B-cell count of 0.5 cells/ml (range 0–2) compared to a median of 24 cells/ml (range 8–46) for Group II patients ( $n = 21$ ) ( $p < 0.0001$ ). The age of onset was comparable between the two groups (Fig. 2). Group I comprised 53% of the entire cohort which is somewhat lower than that reported in adult

populations which ranged from 60–100% (20, 22, 23, 26, 27).

Median serum IgG and IgM were slightly lower among Group I patients compared to Group II patients prior to initiation of treatment with replacement immunoglobulin (345 mg/dl [range 7–471] vs. 381 mg/dl [range 317–469],  $p = 0.06$  and 22 mg/dl [range 4–121] vs. 38 mg/dl [range 18–117],  $p = 0.08$ , respectively). Group I patients also had lower median IgA levels (13 mg/dl [range 6–85] vs. 41 mg/dl [range 8–613],  $p = 0.05$ , respectively). For these analyses, children less than 5 yr of age were excluded to reduce developmental variables.

Group I patients had a lower median number of peripheral  $CD19^+$  B cells compared to Group II patients (232 cells/ml [range 6–651] vs. 553 cells/ml [range 84–1617],  $p = 0.001$ , respectively). This may be seen in Fig. 1, where the patient with low switched memory B cells had fewer CD19 cells. The Group I cohort also demonstrated lower median numbers of  $CD3^+$  T cells and  $CD4^+$  T cells compared to Group II cohort (915 cells/ml [range 330–2613] vs. 1892 cells/ml [range 922–3663],  $p = 0.003$ ; and 611 cells/ml [range 159–1490] vs. 1095 cells/ml [range 606–2636],  $p = 0.006$ , respectively) but equivalent populations of  $CD8^+$  T cells (411 cells/ml [range 112–1282] vs. 633 cells/ml [range 130–1269],  $p = 0.22$ , respectively).

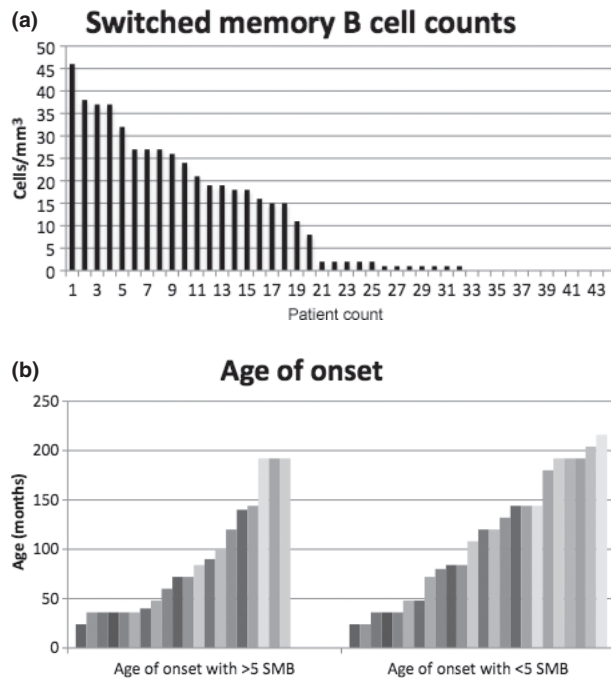


Fig. 2. B-cell counts in pediatric patients with common variable immunodeficiency. (a) Each patient's switched memory B-cell counts are arrayed on the X-axis. There is a bimodal distribution. (b) The patients with greater than or less than five switched memory B cells (SMB) have comparable ages of onset. Each bar represents a single patient, and age is displayed in months on the Y-axis.

Table 1. Baseline characteristics of children with common variable immunodeficiency with (Group I) and without (Group II) low numbers of switched memory B cells

| Characteristic                                                                      | Number (%)       |                   | p-value |
|-------------------------------------------------------------------------------------|------------------|-------------------|---------|
|                                                                                     | Group I (n = 24) | Group II (n = 21) |         |
| Gender                                                                              |                  |                   |         |
| Male                                                                                | 14 (58%)         | 16 (76%)          | 0.34    |
| Age at diagnosis*                                                                   | 84 (24–228)      | 72 (24–192)       | 0.25    |
| Race                                                                                |                  |                   |         |
| White                                                                               | 23 (95%)         | 21 (100%)         | 1.00    |
| Immunologic data*                                                                   |                  |                   |         |
| IgG, mg/dl                                                                          | 345 (7–471)      | 381 (320–469)     | 0.06    |
| IgM, mg/dl                                                                          | 22 (4–121)       | 38 (18–117)       | 0.08    |
| IgA, mg/dl                                                                          | 13 (6–85)        | 41 (8–613)        | 0.05    |
| CD19 <sup>+</sup> B cells, cell/ul                                                  | 232 (6–651)      | 553 (84–1617)     | 0.001   |
| CD19 <sup>+</sup> 27 <sup>+</sup> IgM <sup>+</sup> switched memory B cells, cell/ul | 0.5 (0–2)        | 24 (8–46)         | <0.0001 |
| CD3 <sup>+</sup> T cells, cell/ul                                                   | 915 (330–2613)   | 1892 (922–3663)   | 0.003   |
| CD3 <sup>+</sup> 4 <sup>+</sup> T cells, cell/ul                                    | 611 (159–1490)   | 1095 (606–2636)   | 0.006   |
| CD3 <sup>+</sup> 8 <sup>+</sup> T cells, cell/ul                                    | 411 (112–1282)   | 633 (130–1269)    | 0.22    |

\*Data presented as median (range).

#### Clinical features

The majority of patients in Groups I and II were men (58% vs. 76% [ $p = 0.21$ ], respectively) and Caucasian (95% vs. 100.0%, respectively).

Group I patients had a median age of diagnosis of 84 months of age (range 24–228) compared to 72 months (range 24–192) for Group II patients ( $p = 0.25$ ). There are three patients in this study who have similarly affected siblings. Only one patient has a sibling reported.

Infections were common in both groups. Patients in Group I uniformly had histories of infections compared to 90% of patients in Group II (Table 2). Sinopulmonary infections, including pneumonia, otitis media, and sinusitis, were the most frequent type of infections in both groups. Only Group I patients had meningitis and sepsis.

Autoimmune conditions, while generally rare in this pediatric cohort, occurred exclusively among Group I patients. In aggregate, autoimmune cytopenias were seen in 21% of the Group I patients and none of the Group II patients ( $p = 0.05$ ). These included autoimmune hemolytic anemia in 8%, immune thrombocytopenic

Table 2. Frequency of infectious and non-infectious complications of common variable immunodeficiency in children with (Group I) and without (Group II) low numbers of switched memory B cells

| Characteristic                  | Number (%)       |                   | p-value |
|---------------------------------|------------------|-------------------|---------|
|                                 | Group I (n = 24) | Group II (n = 21) |         |
| Infectious                      |                  |                   |         |
| Otitis media                    | 12 (50%)         | 8 (38%)           | 0.55    |
| Sinusitis                       | 12 (50%)         | 8 (38%)           | 0.55    |
| Pneumonia                       | 11 (46%)         | 10 (47%)          | 1.00    |
| Meningitis                      | 1 (4%)           | 0 (0%)            | 1.00    |
| Sepsis                          | 3 (12%)          | 0 (0%)            | 0.23    |
| Autoimmune                      |                  |                   |         |
| Any autoimmune cytopenia        | 5 (21%)          | 0 (0%)            | 0.05    |
| Neutropenia                     | 2 (8%)           | 0 (0%)            | 0.49    |
| Hemolytic anemia                | 2 (8%)           | 0 (0%)            | 0.49    |
| Immune thrombocytopenic purpura | 2 (8%)           | 0 (0%)            | 0.49    |
| Wegener's granulomatosis        | 1 (4%)           | 0 (0%)            | 1.00    |
| Gastrointestinal                |                  |                   |         |
| Chronic diarrhea                | 3 (12%)          | 0 (0%)            | 0.23    |
| Celiac disease                  | 0 (0%)           | 0 (0%)            | –       |
| Intestinal lymphoid hyperplasia | 1 (4%)           | 0 (0%)            | 1.00    |
| Inflammatory bowel disease      | 0 (0%)           | 0 (0%)            | –       |
| Atrophic gastritis              | 0 (0%)           | 0 (0%)            | –       |
| Protein-losing enteropathy      | 0 (0%)           | 0 (0%)            | –       |
| Pulmonary                       |                  |                   |         |
| Bronchiectasis                  | 1 (4%)           | 0 (0%)            | 1.00    |
| Granulomatous disease           | 2 (8%)           | 0 (0%)            | 0.49    |
| Asthma                          | 7 (29%)          | 8 (38%)           | 0.54    |
| Oncologic                       |                  |                   |         |
| Leukemia                        | 1 (4%)           | 0 (0%)            | 1.00    |
| Lymphoma                        | 1 (4%)           | 0 (0%)            | 1.00    |
| Melanoma                        | 1 (4%)           | 0 (0%)            | 1.00    |
| Lymphatic                       |                  |                   |         |
| Lymphadenopathy                 | 1 (4%)           | 0 (0%)            | 1.00    |
| Splenomegaly                    | 1 (4%)           | 0 (0%)            | 1.00    |
| Atopic                          |                  |                   |         |
| Eczema                          | 4 (17%)          | 1 (5%)            | 0.35    |
| Eosinophilic esophagitis        | 0 (0%)           | 3 (14%)           | 0.09    |

purpura in 8%, and autoimmune neutropenia in 8%. One patient had Evan's syndrome. Both groups rarely exhibited gastrointestinal manifestations of CVID. Three patients in Group I suffered from chronic diarrhea of unknown etiology; however, none of the patients in the study developed celiac disease, inflammatory bowel disease, atrophic gastritis, or protein-losing enteropathy. One patient in Group I has intestinal nodular hyperplasia.

Only those in Group I had evidence of significant chronic pulmonary disease. One patient in Group I developed bronchiectasis, and two patients were diagnosed with granulomatous lung disease. Malignancies, while infrequent, were seen exclusively in Group I. In one patient, the leukemia predated the CVID by several years, and in the other, the lymphoma occurred 10 yr after diagnosis of CVID. One patient with malignant melanoma was identified. None of the patients had any documented splenomegaly or lymphadenopathy. Asthma was seen more slightly more frequently among Group II patients (38% vs. 29%,  $p = 0.54$ ), but eczema was seen more commonly among Group I patients (17% vs. 5%,  $p = 0.35$ ). Eosinophilic esophagitis was seen exclusively in Group II patients.

## Discussion

Our study on pediatric patients with CVID found that those with few or absent  $CD19^+27^+IgM^-$  switched memory B cells exhibit a different immunologic phenotype than those with higher numbers of switched memory B cells. Those in the former group, Group I, had lower serum IgG, IgM, and IgA levels compared to Group II patients, although only the IgA level reached statistical significance. They also had significantly lower numbers of  $CD19^+$  B cells. Patients in Group I had evidence of more significant infections (e.g., sepsis and meningitis), autoimmune disease (e.g., autoimmune hemolytic anemia and immune thrombocytopenic purpura), bronchiectasis, granulomatous lung disease, and hematologic malignancies.

This pediatric CVID cohort has some important similarities with studies of adult patients with CVID. Prior studies on adults documented lower levels of serum IgG in those with low numbers of switched memory B cells (7, 27). Prior studies also found higher rates of autoimmune and granulomatous disease in patients with low numbers of switched memory B cells (7, 22, 27, 33). The largest adult study found that 14% of patients with low switched memory B cells had autoimmune cytopenias, 13% had granuloma-

tous disease, and 39% had splenomegaly (23). Splenomegaly stands out as being significantly higher in the adult literature. We also found that Group I patients also had histories of more serious infections, such as meningitis and sepsis, bronchiectasis, and lymphoproliferative disease compared to those in Group II; however, the differences in frequency of these phenotypic manifestations of CVID were not statistically significant. This study suggests that the relationship between numbers of switched memory B cells and rare, potentially fatal manifestations of CVID should be explored in future research in larger cohorts of pediatric patients with CVID as it has important implications for prognosis (10).

This study has several limitations. As an observational study, we cannot infer a causal relationship between the number of switched memory B cells in pediatric patients with CVID and their immunologic and clinical phenotype. The severity of illness of the patients evaluated at our center may be dissimilar from those seen in community settings. Finally, the sample size limits our ability to assess for small differences in immunologic and clinical phenotypes in this patient population. However, to our knowledge, this study describes the largest assessment of pediatric patients with CVID focusing on switched memory B cells.

In our cohort, only those pediatric patients with low numbers of switched memory B cells exhibited evidence of autoimmune disease and granulomatous disease as well as more frequent complications, including serious infections and pulmonary disease. Long-term clinical follow-up of larger cohorts of pediatric patients with CVID will be essential to understand the relationship between B cell subsets and disease manifestations – both immunologically and phenotypically – and the ultimate utility of memory B-cell assessments in disease management and prognosis. The pediatric cohort described in this study is largely similar to adult cohorts, although the time of disease burden is shorter and overall end organ damage may be less. Our cohort had a comparable frequency of autoimmune disease compared to adult cohorts (7, 15, 23). This finding suggests that pediatric CVID represents one end of the age spectrum and not a distinct disease as had been previously suggested. The pediatric CVID population may warrant more aggressive intervention because of their potential for a longer disease duration and higher disease burden. As the Group I population appears to have a higher prevalence of infections, autoimmune and lymphoproliferative complications, it may be prudent to target more aggressive surveillance

and interventions to this subgroup. Additional studies may allow the development of specific recommendations regarding surveillance and management of the high-risk population.

### Acknowledgments

Dr. Pierre Yong is supported by a training grant from the Philadelphia Veterans Affairs Medical Center and the Robert Wood Johnson Clinical Scholars Program at the University of Pennsylvania. We are grateful for the assistance of Benjamin Garcia, who helped with data management for this project and the assistance of Sarah Shaw, Joe McMann, and USIDNET.

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