

Letter to the Editor

Serum free light chains in the differential diagnosis and prognosis of primary and secondary hypogammaglobulinemia

To the Editor:

In adult patients the diagnosis of common variable immunodeficiency (CVID) is often an exclusion process, and lymphoproliferative disorders (LPDs) or plasma cell dyscrasias (PCDs) have to be carefully ruled out. Differential diagnosis might represent a real challenge because of similar clinical aspects. In adults with antibody deficiency, a bone marrow examination might be required to rule out hematologic malignancies. The serum free light chain (sFLC) assay is widely used in patients with LPDs and PCDs to obtain prognostic information on the disease's clinical course.^{1,2} Because primary antibody deficiency (PAD) has been associated with a low production of light chains,³ in this retrospective study we evaluated the role of sFLCs in the differential diagnosis between patients with primary and secondary antibody defects.

Clinical data and sFLC levels were collected from clinical records of 45 patients with CVID, 10 patients with other PADs, and 44 patients initially referred because of hypogammaglobulinemia, in which a secondary antibody deficiency resulted as final diagnosis (patients' demographic and clinical details are summarized in the [Methods](#) section and [Table E1](#) in this article's Online Repository at www.jacionline.org). All patients with CVID fulfilled the European Society for Immunodeficiencies/Pan-American Group for Immunodeficiency diagnostic criteria.⁴

On the basis of sFLC levels, patients with CVID were classified into 4 groups ([Fig 1](#)): in 3 groups κ ($\kappa^+ \lambda^-$ pattern, 24/45 patients), λ ($\kappa^- \lambda^+$ pattern, 1/45 patients), or both ($\kappa^+ \lambda^+$ pattern, 15/45 patients) light chains were reduced or undetectable. We defined these 3 patterns as CVID-like because only 5 of 45 patients presented with normal sFLC levels ($\kappa^+ \lambda^+$). In our cohort the sensitivity of the CVID-like pattern of sFLCs was 89% (95% CI, 76% to 96%), and the specificity was 100% (95% CI, 92% to 100%). By using this pattern as a diagnostic marker for CVID, the positive predictive value was 100% (95% CI, 92% to 100%), and the negative predictive value was 90% (95% CI, 78% to 97%). Measurement of sFLC levels over the years revealed superimposable levels of both chains in each patient not influenced by replacement therapy with immunoglobulins; this suggests a diagnostic value of the test, even in patients in whom replacement therapy has been started to prevent infections. It is intriguing that in all patients with CVID, κ and λ chains, as determined by using flow cytometric analysis, were normally expressed on B-cell surface immunoglobulins (mean surface κ/λ ratio, 1.23 ± 0.23). Concerning other kinds of PADs, in 2 of 2 patients with X-linked agammaglobulinemia, sFLC levels resulted undetectable. In 1 of 7 patients with IgG subclass deficiency, we found a low κ light chain level, whereas in the other 6 patients and in 1 patient with IgA deficiency, levels of both chains were normal.

None of the 44 patients with secondary antibody deficiency presented with a CVID-like pattern on the sFLC assay ([Fig 1](#)). The most common finding in patients with LPDs was a normal level of both κ and λ chains, as previously described in larger

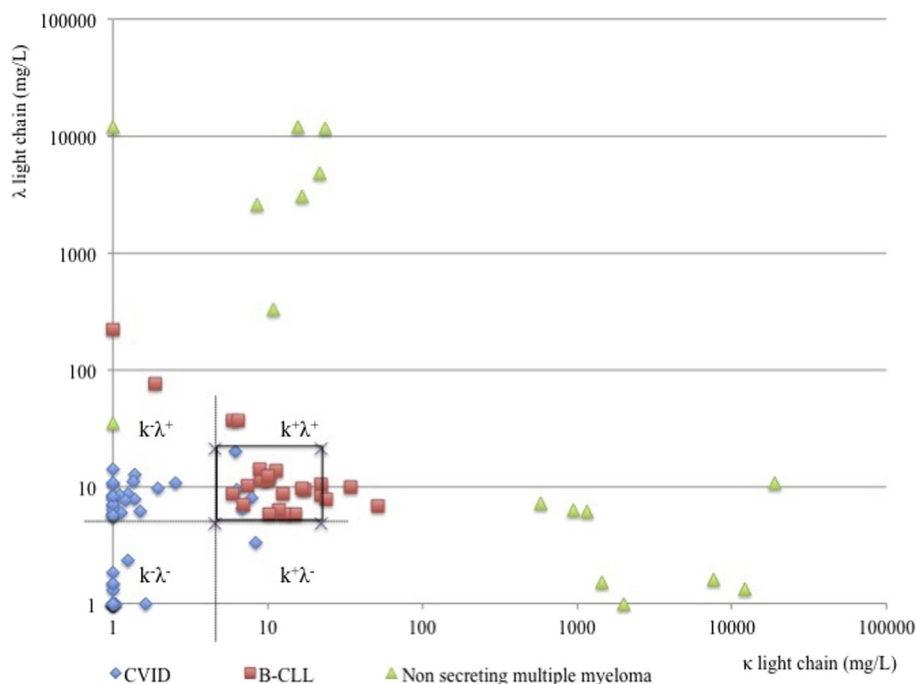


FIG 1. sFLC levels in the differential diagnosis of hypogammaglobulinemia. The scatter plot shows sFLC levels for 45 patients with CVID and 44 patients with secondary antibody deficiency. Patients with CVID have an sFLC pattern different from that of patients with secondary hypogammaglobulinemia. sFLC patterns in patients with CVID are divided by *dashed lines*, as indicated. The *square* represents reference sFLC values. B-CLL, B-cell chronic lymphocytic leukemia.

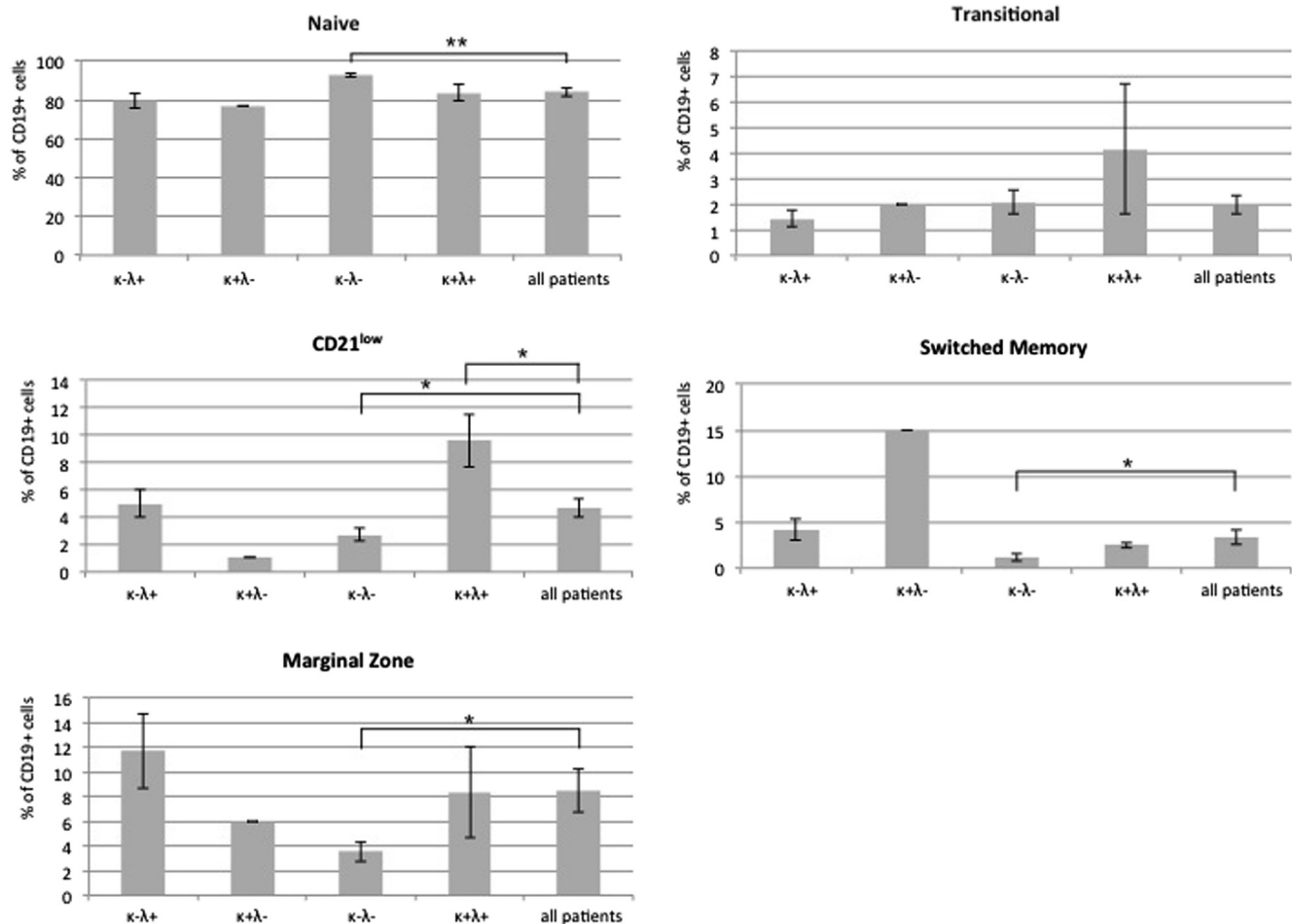


FIG 2. B-cell subpopulations in the 4 sFLC subgroups and the whole CVID cohort (means and SEs). sFLC patterns seem to be able to differentiate between biological subgroups of the disease. Because only 1 patient presented with a $\kappa^+\lambda^-$ pattern, we did not look for significant differences in B-cell subtypes for this group. * $P < .05$ and ** $P < .01$.

cohorts.² In all patients with PCDs, we found a higher level for either κ or λ chains. We detected normal sFLC levels in 3 patients with other secondary forms of hypogammaglobulinemia. Our data indicate that the evaluation of sFLC levels is useful to exclude LPDs, PCDs, and other common causes of hypogammaglobulinemia in patients with a suspected PAD.

Next, we compared the diagnostic power of bone marrow biopsy (BMB) specimens in relationship to sFLC levels in patients with both primary and secondary hypogammaglobulinemias. Among the 99 patients, a BMB was necessary in 59 during the diagnostic workup. In 41 of these patients, a diagnosis of lymphoid malignancy was made, whereas in 18 the final diagnosis was CVID. In all 14 patients with the CVID-like pattern of sFLCs, the BMB specimen was normal, whereas in 41 (91%) of 45 patients with a non-CVID-like pattern, a diagnosis of LPD was made (see Table E2 in this article's Online Repository at www.jacionline.org). Thus the CVID-like pattern of sFLCs showed a complete diagnostic concordance with the results of BMBs. These data suggest a possible role of the sFLC assay in deciding whether to perform invasive investigations in patients with hypogammaglobulinemia and suspected hematologic malignancies.

Once a diagnosis of CVID is made, the clinical heterogeneity of these patients requires their classification into distinct clinical phenotypes to better predict different clinical behaviors and prognoses.⁵ Several disturbances in B-cell homeostasis have also been observed in patients with CVID.⁶⁻⁸ Even if our small cohort does not allow firm conclusions but only suggestions, it is interesting to note the correlations we have found between sFLC levels and clinical and flow cytometric phenotypes (see the Methods section and Tables E3-E5 in this article's Online Repository at www.jacionline.org). In the most represented sFLC pattern ($\kappa^-\lambda^+$), the majority of patients (12 [57%] of 21, $P = .14$) presented with one of the clinical phenotypes associated with higher risk of mortality (ie, autoimmune cytopenias, benign lymphoproliferation, and enteropathy).⁵ Patients with the $\kappa^-\lambda^-$ pattern tended to present with splenomegaly (6 [40%] of 15 patients, $P = .13$) and malignancies (5 [33%] of 15 patients, $P = .11$). Flow cytometric data showed a significant increase in the percentage of naive B cells and a significant decrease in numbers of switched memory, marginal zone, CD21^{low} B cells (Fig 2). Thus the $\kappa^-\lambda^-$ pattern seems to be characterized by normal development of naive and transitional B cells and a marked

decrease of the subsets linked to B-cell activation and immunoglobulin production. We detected malignancies in 3 (60%) of 5 patients with a $\kappa^+\lambda^+$ pattern, and this association resulted in just over the limits of statistical significance ($P = .06$). Autoimmune diseases were reported in 3 of these patients (autoimmune gastritis, psoriasis, and Sjogren syndrome). Interestingly, flow cytometric data displayed a significant increase in the percentage of CD21^{low} B cells, cells that have been found to be enriched in patients with CVID and autoimmunity.⁹ Almost all cases of malignancies (8 [89%] of 9) were detected in patients with a $\kappa^+\lambda^+$ or $\kappa^-\lambda^-$ pattern ($P < .01$).

Taken together, our data suggest that the sFLC assay could represent a promising marker to help in the diagnosis and risk stratification of adults with CVID, identifying subgroups characterized by peculiar biological characteristics, clinical behavior, and prognosis. Further studies in wider cohorts are needed to confirm our preliminary results.

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METHODS

In this retrospective study clinical data and sFLC levels were extracted from clinical records of 55 patients with PADs. Specifically, data were collected from 45 patients with CVID, 2 patients with X-linked agammaglobulinemia (diagnosis based on genetic tests), 7 patients with IgG subclass deficiency, and 1 patient with IgA deficiency. All patients with CVID fulfilled the European Society for Immunodeficiencies/Pan-American Group for Immunodeficiency diagnostic criteria, including a marked decrease in IgG levels (≥ 2 SDs below the mean for age), a marked decrease in at least 1 of the isotypes IgM or IgA, onset of clinical significant immunodeficiency at an age greater than 2 years, and exclusion of defined causes of hypogammaglobulinemia.

We included only adult patients in whom the sFLC assay had been performed at diagnosis and at least once during follow-up. We collected up to 4 doses over 3 years in some of them (normal references in our hospital laboratory: λ chain, 4,840–21,880 mg/L; κ chain, 4,520–22,330 mg/L). The condition in which either the κ chain, λ chain, or both were reduced was defined as a CVID-like pattern. All subjects included in the study were divided into 4 groups according to sFLC levels: the $\kappa^- \lambda^+$ pattern, in which a reduction of the κ light chain only was observed; the $\kappa^+ \lambda^-$ pattern, in which a reduction of the λ light chain only was observed; the $\kappa^- \lambda^-$ pattern, which was characterized by a reduction in levels of both light chains; and the $\kappa^+ \lambda^+$ pattern, which was characterized by normal sFLC levels. We excluded from our analysis 3 patients in whom we detected different sFLC patterns in the repeated dosages.

A clinical history was collected from all patients with CVID, and patients were divided into subgroups according to the revised version of clinical phenotypes proposed by Chapel et al.^{E1} In addition, the presence of splenomegaly, malignancies, and bronchiectasis was also analyzed. In 38 of 45 patients, a flow cytometric analysis was performed to detect levels of CD19⁺ cells, switched memory B cells (IgM⁺IgD⁺CD27⁺), marginal zone B cells (IgM⁺IgD⁺CD27⁺), CD21^{low} B cells (CD38^{low}CD21^{low}), transitional B cells (CD38^{hi}IgM^{hi}), and plasmablasts (CD38⁺⁺⁺IgM⁺), according to the methods described in the EUROclass trial.^{E2}

We also collected sFLC levels from the clinical records of 44 patients initially referred to our outpatient clinic because of hypogammaglobulinemia, in which a secondary antibody deficiency resulted as final diagnosis: 25 patients with untreated/pre-treatment chronic lymphocytic leukemia, 16 patients with a new diagnosis of nonsecreting multiple myeloma, 1 patient with hypogammaglobulinemia secondary to prolonged high-dosage steroid therapy, 1 patient with a protein-losing enteropathy, and 1 patient with nephrotic syndrome. We included only patients in whom hypogammaglobulinemia was present at disease onset, and the sFLC assay was performed before any treatment.

Summary statistics (sensitivity, specificity, positive predictive value, negative predictive value, and 95% CI) were computed to compare sFLC results in patients with CVID and secondary hypogammaglobulinemia. The

Fisher exact test was used to assess correlation between CVID clinical phenotypes and sFLC patterns. The unpaired *t* test (with the Welch correction, when needed, according to the F test) was used to compare the percentage of B-cell subpopulations among the 4 sFLC groups.

Informed consent was obtained from all patients, and the study was approved by the local research ethics committee.

Patients

CVID. Forty-five patients (26 male and 19 female patients) with CVID were included in this study. The mean age at diagnosis was 39 years (range, 16–63 years). At diagnosis, mean immunoglobulin levels were 323 mg/dL for IgG, 27 mg/dL for IgA, and 35 mg/dL for IgM. Data sufficient for further clinical phenotyping were available for 42 of 45 patients. Autoimmune cytopenias were present in 6 (14%) patients. Eight (19%) patients had polyclonal lymphocytic infiltration. We detected CVID-associated enteropathy in 4 (10%) patients. Twenty-six (62%) patients had a “no other disease-related complication” phenotype. In addition, the long-term consequences of recurrent infections in the form of bronchiectasis were seen in 10 (24%) patients, 9 (21%) patients presented with lymphoid and nonlymphoid malignancies, and 10 (24%) patients had splenomegaly. As previously described by Chapel et al,^{E1} only a minority of patients (2.5%) matched the criteria for 2 clinical phenotypes. Patients' clinical phenotypes are summarized in Table E1; associations between clinical phenotypes and sFLC patterns are summarized in Tables E3 and E4.

Secondary immunodeficiency with hypogammaglobulinemia. Forty-four patients (27 male and 17 female patients) were included who were initially referred to our outpatient clinic because of hypogammaglobulinemia, in which a secondary antibody deficiency resulted as final diagnosis: 25 patients with untreated/pre-treatment chronic lymphocytic leukemia, 16 patients with a new diagnosis of nonsecreting multiple myeloma, 1 patient with hypogammaglobulinemia caused by a prolonged high-dosage steroid therapy, 1 patient with a protein-losing enteropathy, and 1 patient with nephrotic syndrome. We included only patients in which hypogammaglobulinemia was present at the onset of the disease, and the sFLC assay was performed before any treatment. The mean age at diagnosis was 58 years (range, 38–82 years). At diagnosis, mean immunoglobulin levels were 502 mg/dL for IgG, 59 mg/dL for IgA, and 25 mg/dL for IgM.

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TABLE E1. Clinical details of patients with CVID

Characteristics of patients (n = 42)	
Cytopenias, no. (%)	6 (14)
Polyclonal lymphoproliferation, no. (%)	8 (19)
Unexplained persistent enteropathy, no. (%)	4 (10)
No other disease-related complications, no. (%)	26 (62)
Bronchiectasis, no. (%)	10 (24)
Splenomegaly, no. (%)	10 (24)
Malignancy, no. (%)	9 (21)

TABLE E2. sFLC and bone marrow examination in the differential diagnosis of hypogammaglobulinemia

	sFLC pattern			
	CVID-like pattern (n = 14)		Non-CVID-like pattern (n = 45)	
Final diagnosis	CVID	14 (100%)	CVID	4 (9%)
	LPDs or MM	0 (0%)	LPDs or MM	41 (91%)

MM, Multiple myeloma.

TABLE E3. sFLC patterns and clinical phenotypes

sFLC pattern	Clinical phenotype*				Complications		
	1	2	3	4	Splenomegaly	Malignancy	Bronchiectasis
$\kappa^{-}\lambda^{+}$ (n = 21)	9 (42%)	5 (24%)	5 (24%)	2 (10%)	3 (14%)	1 (5%)	6 (29%)
$\kappa^{+}\lambda^{-}$ (n = 1)	1 (100%)	—	—	—	—	—	—
$\kappa^{-}\lambda^{-}$ (n = 15)	10 (67%)	1 (7%)	2 (13%)	2 (13%)	6 (40%)	5 (33%)	2 (13%)
$\kappa^{+}\lambda^{+}$ (n = 5)	4 (80%)	—	1 (20%)	—	1 (20%)	3 (60%)	3 (60%)

*1, No other disease-related complications; 2, cytopenias; 3, polyclonal lymphoproliferation; 4, unexplained persistent enteropathy.

TABLE E4. sFLC patterns and malignancies

sFLC pattern	Malignancy	
	No. of cases	Diagnosis
$\kappa^{-}\lambda^{+}$	1 (5%)	Gastric cancer (n = 1)
$\kappa^{+}\lambda^{-}$	5 (33%)	Gastric cancer (n = 2), NHL (n = 2), breast cancer (n = 1)
$\kappa^{-}\lambda^{-}$		
$\kappa^{+}\lambda^{+}$	3 (60%)	Colorectal cancer (n = 2), paraganglioma (n = 1)

NHL, Non-Hodgkin lymphoma.

TABLE E5. B-cell subpopulations in the 4 sFLC subgroups and the whole CVID cohort

sFLC pattern	B-cell subpopulations									
	Naive		Transitional		CD21 ^{low}		Switched memory		Marginal zone	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
$\kappa^{-}\lambda^{+}$	79.32	3.74	1.45	0.34	5.01	1.03	4.24	1.20	11.74	3.06
$\kappa^{+}\lambda^{-}$	77.00	0.00	2.00	0.00	1.00	0.00	15.00	0.00	6.00	0.00
$\kappa^{-}\lambda^{-}$	92.97	1.22	2.07	0.46	2.73	0.46	1.15	0.43	3.56	0.81
$\kappa^{+}\lambda^{+}$	83.78	4.50	4.18	2.54	9.60	1.92	2.48	0.26	8.40	3.72
All patients	84.19	2.31	1.99	0.38	4.70	0.70	3.35	0.77	8.53	1.78

sFLC patterns seem to be able to differentiate between biological subgroups of the disease.