

Letter to the Editor

Confirmation and improvement of criteria for clinical phenotyping in common variable immunodeficiency disorders in replicate cohorts

To the Editor:

The clinical diversity of common variable immunodeficiency disorders (CVIDs) required division of these patients into distinct clinical phenotypes to provide more homogeneous groups for immunopathologic studies as well as individual patient prognosis. By using data on clinical complications and disease progression from 334 patients with CVID from 7 European centers including Oxford ("Northern European cohort"), distinct clinical phenotypes were defined with relatively little overlap: 83% of the patients had only 1 clinical phenotype.¹ The prognostic significance of the defined phenotypes was confirmed by significant correlation with survival.^{1,2}

Comparison of data in 2 independent databases (DEFI and Mount Sinai) (see this article's Online Repository at www.jacionline.org) showed that it was feasible to confirm clinical phenotyping criteria in replicate cohorts, using identical definitions for each clinical complication and laboratory finding. In addition, further associations were sought to improve the criteria for clinical phenotyping and survival. Predictors of clinical phenotypes were previously limited to IgM serum levels for the lymphoproliferative (LP) phenotype and malignancy as well as low peripheral CD8 counts for autoimmunity. Further searches for predictive laboratory measurements were undertaken on the combined cohorts.

Since the dates of diagnosis for complications in patients in 2 cohorts (Oxford and the DEFI study) were known, the interval from the diagnoses of CVID and the first disease-related complication was calculated to search for the earliest date for reliable prognosis.

Initially, it was important to confirm the concept of clinical phenotyping in CVIDs. The proportions of patients in the independent replicate cohorts with features of only 1 clinical phenotype were similar between cohorts when the original criteria were used (see Fig E1 in this article's Online Repository at www.jacionline.org). By using the discovery (Northern European; $n = 334$) cohort, further associations between complications were found to improve the phenotyping criteria. Considering the LP phenotype, no significant correlation was found between granuloma or lymphoid interstitial pneumonitis (LIP) and hepatomegaly, and so hepatomegaly was discarded. Lymphadenopathy remained as a criterion for LP, since although only 1 patient of 11 in the extended Oxford cohort did not also have granuloma or LIP, there were 29 patients with persistent lymphadenopathy in the Mount Sinai cohort of whom 10 had no obvious symptoms of granuloma and/or LIP. When autoimmunity was split into 2 categories, autoimmune cytopenias and organ-specific autoimmune diseases, only cytopenias showed decreased survival ($P = .0001$); organ-specific autoimmunity was not associated with cytopenias or the other clinical phenotypes. Lymphoid malignancies were excluded in the revised phenotyping criteria since CVID may not be the primary event.²

Thus, the revised phenotyping criteria were as follows:

- no other disease-related complications (previously "infections only")

- cytopenias (thrombocytopenia/autoimmune hemolytic anemia/neutropenia)
- polyclonal lymphoproliferation (granuloma/LIP/persistent unexplained lymphadenopathy)
- unexplained persistent enteropathy.

Using the new phenotyping criteria (see Table E1 in this article's Online Repository at www.jacionline.org for comparison), the proportions of those with only 1 phenotype in the 3 cohorts now ranged from 89% to 94% (Fig 1).

Previously, there were center and country variations in the proportions of individual complications¹; this was confirmed in this larger study. There was a higher proportion of patients with no disease-related complications ("infections only") phenotype in the discovery (Northern Europe) cohort (78%) than in either of the confirmatory cohorts (DEFI 66% and Mount Sinai 63%). The New York cohort had more cytopenic patients, and the DEFI group had more patients with polyclonal lymphoproliferation, among both the single phenotype patients and those with 2 clinical phenotypes (Fig 1). These differences may relate to the intrinsic bias of referrals to each center or real differences in the genetic makeup or environmental exposure of patients. There were insufficient numbers to divide into groups by ethnicity (those patients with a combined deficiency known as late-onset combined immunodeficiency related to consanguinity had been excluded³).

The data relating to the date of onset of disease-related complications were available in the DEFI and extended Oxford cohorts ($n = 453$). This was analyzed to find the stage in disease progression at which a clinical phenotype can be determined (see Fig E2 in this article's Online Repository at www.jacionline.org). Most cytopenias (77%) presented before or at CVID diagnosis. In contrast, unexplained enteropathy was present in only 3 of the 26 patients at this stage. The proportion of patients with polyclonal lymphoproliferation after diagnosis was approximately equal to the number before or at diagnosis. The data also showed that few patients developed phenotype-defining complications more than 15 years following diagnosis. Of the 41 patients in these cohorts who developed 2 disease-related complications, only 5 (12%) developed the second phenotype-defining complication more than 14 years after the CVID diagnosis. However, hazard plots showed that the probability of developing each of the phenotypes at 5 years after diagnosis was not statistically different from that at 15 years, suggesting that prognosis was significantly secure 5 years after CVID diagnosis for 95% of the patients.

The combined cohorts were used to find indicators for the clinical phenotypes ($n = 848$) (a full list is given in Tables E2-E5 in this article's Online Repository at www.jacionline.org). In the original study, only initial IgM serum levels were predictive for the LP phenotype and lymphoid malignancies, whereas the initial CD8 percentages predicted autoimmunity. Subsequent analysis (see Table E1) shows 14% increase in the risk of cytopenia for every 1 g/L increase in IgG; 69% decrease in the odds of LP for every 1 unit increase in IgA; 180% increase in the odds of no disease-related complications for each 1 g/L increase in IgA; 50% increase in the odds of hepatomegaly for each 1 g/L increase in IgM; and 85% decrease in the odds of hepatomegaly for each $1 \times 10^9/L$ increase in CD4 numbers. Once hepatomegaly was

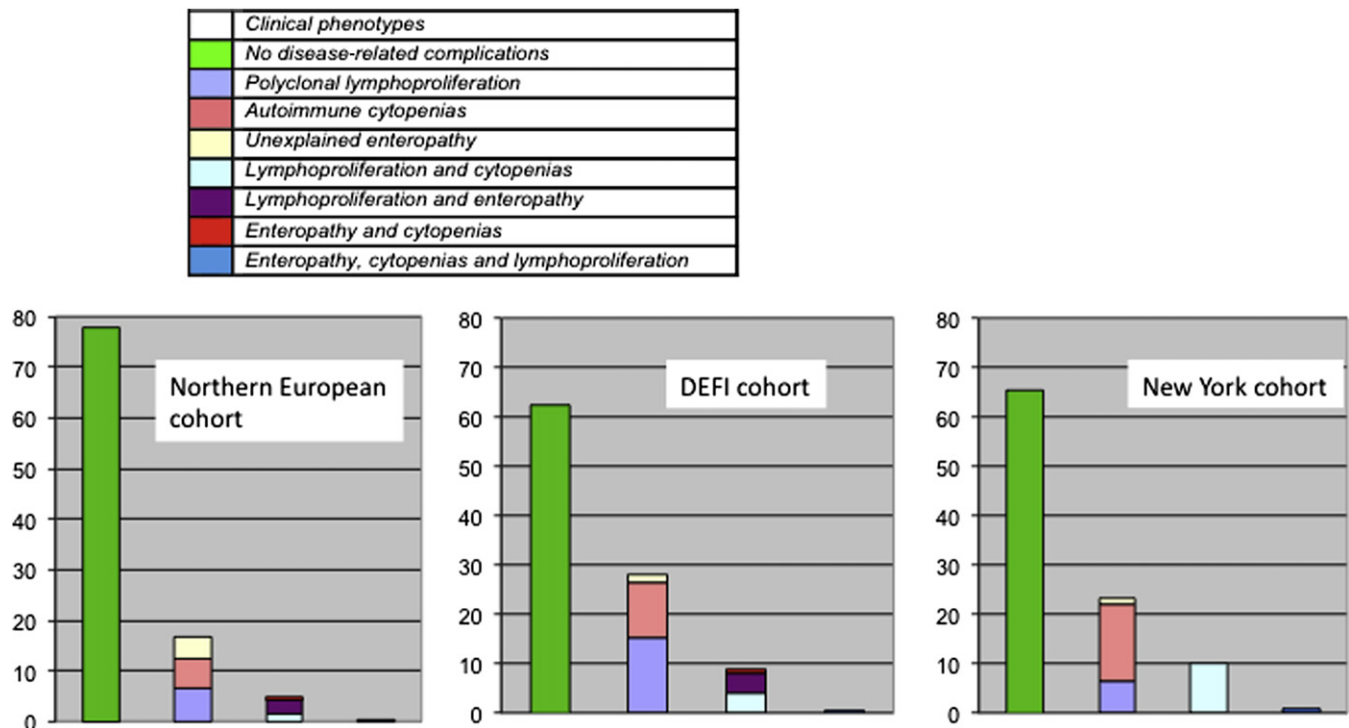


FIG 1. The percentages of patients in each of the 3 cohorts with various numbers of clinical phenotypes.

removed, high initial serum IgM levels were no longer predictive of the lymphoproliferative phenotype, though low serum IgA was related to LP.

Regular β -2 microglobulin levels were measured regularly in the extended Oxford cohort only. Even in this small cohort ($n = 94$) there were associations between raised levels of serum β -2 microglobulin (>3 mg/mL) with splenomegaly ($P < 10^{-6}$), as well as LIP ($P = .0002$), granuloma ($P = .002$), and nodular regenerative hyperplasia ($P = .02$). This is in keeping with active polyclonal lymphocyte turnover whether due to lymphocyte activation or due to infection.⁴

Previously, the survival of patients with particular clinical phenotypes was least good in the enteropathic CVID group, followed by the LP phenotype and then the autoimmune phenotype.¹ Analysis on the combined cohorts ($n = 848$) confirmed this and also showed that cytopenia reduced life expectancy, too (see Table E4). Bronchiectasis and splenomegaly were excluded previously as phenotyping criteria; their contribution to survival was examined by using the combined data. Based on the Cox regression model (which incorporates unequal follow-up time), bronchiectasis was the lesser risk (relative risk = 1.82; $P = .04$) compared with splenomegaly (relative risk = 4.35; $P < .0001$). In terms of the laboratory findings examined (see Table E2), the only poor prognostic indicator was reduced or absent circulating B cells.

The confirmation of clinical phenotyping criteria on separate independent cohorts substantiates the original findings. The phenotyping criteria have been refined to improve the distinction of the 4 clinical phenotypes more efficiently. Furthermore, for the vast majority of patients, it may be possible to provide a prognosis within 5 years of a CVID diagnosis.

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CVIDs are a group of heterogeneous conditions in which failure to produce protective antibodies results in serious or recurrent bacterial infections.^{E1,E2} CVIDs are diagnoses of exclusion when other primary and secondary causes of antibody failure have been excluded. The clinical diversity of these conditions has been known for some time, and there was a need to divide patients according to clinical complications and disease progression. This would provide more homogeneous groups for studying the immunopathogenesis of the commonest symptomatic forms of primary antibody deficiency.

Data to confirm such clinical observations were based on a cohort of 334 patients from 7 European centers (Northern European cohort). Only patients with a validated diagnosis of a CVID were included; 326 (84%) were followed for at least 10 years since the onset of symptoms, and 237 (61%) patients were followed for more than 18 years. The data were used to define 5 clinical phenotypes, with relatively little overlap in that 83% of the patients had only 1 clinical phenotype.^{E1}

The clinical phenotypes were defined as follows:

- no disease-related complications (described as “infections only”)
- autoimmunity (including organ-specific autoimmune diseases and cytopenias)
- polyclonal lymphoproliferation (including hepatomegaly, persistent unexplained lymphadenopathy, lymphoid interstitial pneumonitis, noninfective granuloma)
- unexplained enteropathy (excluding infective, autoimmune, and gluten-sensitive enteropathies)
- lymphoid malignancies.

The prognostic significance of the defined phenotypes was confirmed by significant correlation with survival.^{E1,E2} An attempt to define prognostic indicators was also made though only raised IgM serum levels were predictive of the LP and malignant phenotypes and low CD8 counts in peripheral blood were associated with autoimmunity.

Following the interest in this article, we have sought to provide confirmatory data on replicate cohorts using identical definitions for each clinical complication or laboratory finding. Comparison of the data collections in 2 independent databases showed that this was feasible in terms of compatibility of clinical definitions and length of documented follow-up available.

It was important not only to confirm the original findings but also to look for country or continent differences since the original data from Northern Europe showed significant differences between centers and countries. A further search for prognostic factors was done in those cohorts. Since the dates of the complications in patients in 2 cohorts (Oxford and DEFI study) were known, the interval from the diagnoses of CVID and the first disease-related complication was calculated to search for the earliest date for reliable prognosis.

METHODS

Patients

Clinical and laboratory data from 2 independent cohorts of patients with a validated diagnosis of a CVID were used. The patient cohort from the Department of Clinical Immunology at the Mount Sinai School of Medicine, New York (n = 214),^{E3} and that from The French PID study group: DEFI (n = 335)^{E4} were compared with those from the Northern European cohort (n = 334 patients) by using methodology identical to that used in the original

article.^{E1} The discovery cohort from Northern Europe included 83 patients from Oxford; this cohort has been extended to include 35 additional patients who now qualify in terms of follow-up time, and the cohort is described as the “extended Oxford cohort.”

Diagnosis

The 2008 article used the 1999 definition for CVID,^{E5} including the exclusion of other known B-cell defects. As in the original study, there was rigorous validation of the diagnoses of CVIDs from data within the databases and if there were insufficient data to validate the diagnosis, all the data for that patient were excluded. Since CVID is a diagnosis of exclusion, patients were included only if there was sufficient clinical, laboratory, or genetic data to exclude the following: chromosomal aberrations, renal loss, drug-related hypogammaglobulinemia, thymoma, lymphoid malignancy (development of lymphoid neoplasms within 2 years after the diagnosis of hypogammaglobulinemia), X-linked agammaglobulinemia, genetic aberrations that can be associated with low circulating B cells, such as DNA Ligase4, RAG1 RAG2 (atypical) resulting in leaky forms of severe combined immune deficiencies, the various genetic abnormalities associated with autosomal-recessive agammaglobulinemia resulting in early onset of marked infections, known hyper-IgM syndromes, and syndromic hypogammaglobulinemias.^{E2}

Patients with absent circulating B cells but no *BTK* mutation were included on the basis that they make up 12% of the CVID cohort.^{E1} Since 10% to 15% of male and all the female patients do not have X-linked agammaglobulinemia,^{E6} it was agreed to include these patients if the individual clinical and laboratory data met the CVID diagnostic criteria, as they may represent a distinct group of patients with CVIDs.

Complications

The definition for each clinical complication or laboratory finding was identical to that used in the original publication; these were also agreed in advance with the current authors.

Prognostic

These indicators investigated for clinical phenotype in all 3 cohorts: the number of patients for each indicator is included in the tables.

RESULTS AND DISCUSSION

Confirmation of clinical phenotyping using the original criteria

See Fig E1.

Improving clinical phenotyping criteria. The underlying pathology for hepatomegaly is consistently found to be nodular regenerative hyperplasia in patients with CVID (in the absence of hepatitis C infection).^{E7,E8} This is an unusual condition, though associated with innate and adaptive immune dysregulation is thought to be of vascular origin,^{E9,E10} and so it may not be specifically related to CVID. Removal of lymphoid malignancies as a separate clinical phenotype resulted in the exclusion of 10 of 334 from the Northern European data, 14 of 214 from the New York cohort, and 11 of 335 from the DEFI data.

Time interval between CVID diagnosis and disease-related complications involved in clinical phenotyping

See Fig E2.

Clinical and laboratory findings examined for clinical phenotypes. In the original study, only initial IgM serum levels were predictive for the LP phenotype and lymphoid malignancies whereas the initial CD8 percentages predicted autoimmunity.

Given the revision of the phenotyping criteria, this was revisited by using data from the combined cohorts ($n = 848$) (Table E1).

Iron deficiency. Iron deficiency was present in about a third of patients in most centers, regardless of gender. There was no indication in the extended Oxford cohort that persistent iron deficiency was associated with the enteropathic phenotype or low IgA; neither did persistent iron deficiency have an effect on survival.

To examine the question of the poorer prognosis in children with a diagnosis of CVID before their 16th birthday, clinical phenotypes were compared in patients younger than 16 years with those older than 16 years. The data in Table E2 show that there were more cytopenic phenotypes in the younger group; the distribution of the other clinical phenotypes was similar.

Analysis of the difference in β -2 microglobulin levels (see Table E4) was undertaken by using a linear mixed model for various phenotypes. If the P value was significant, then the unadjusted means and SDs are given for the absence or presence of the phenotype.

Clinical and laboratory findings for survival. Given the different prognoses for the clinical phenotypes in the original data,^{E1} this was reexamined with the revised criteria by using the combined cohorts. Table E5 shows the results.

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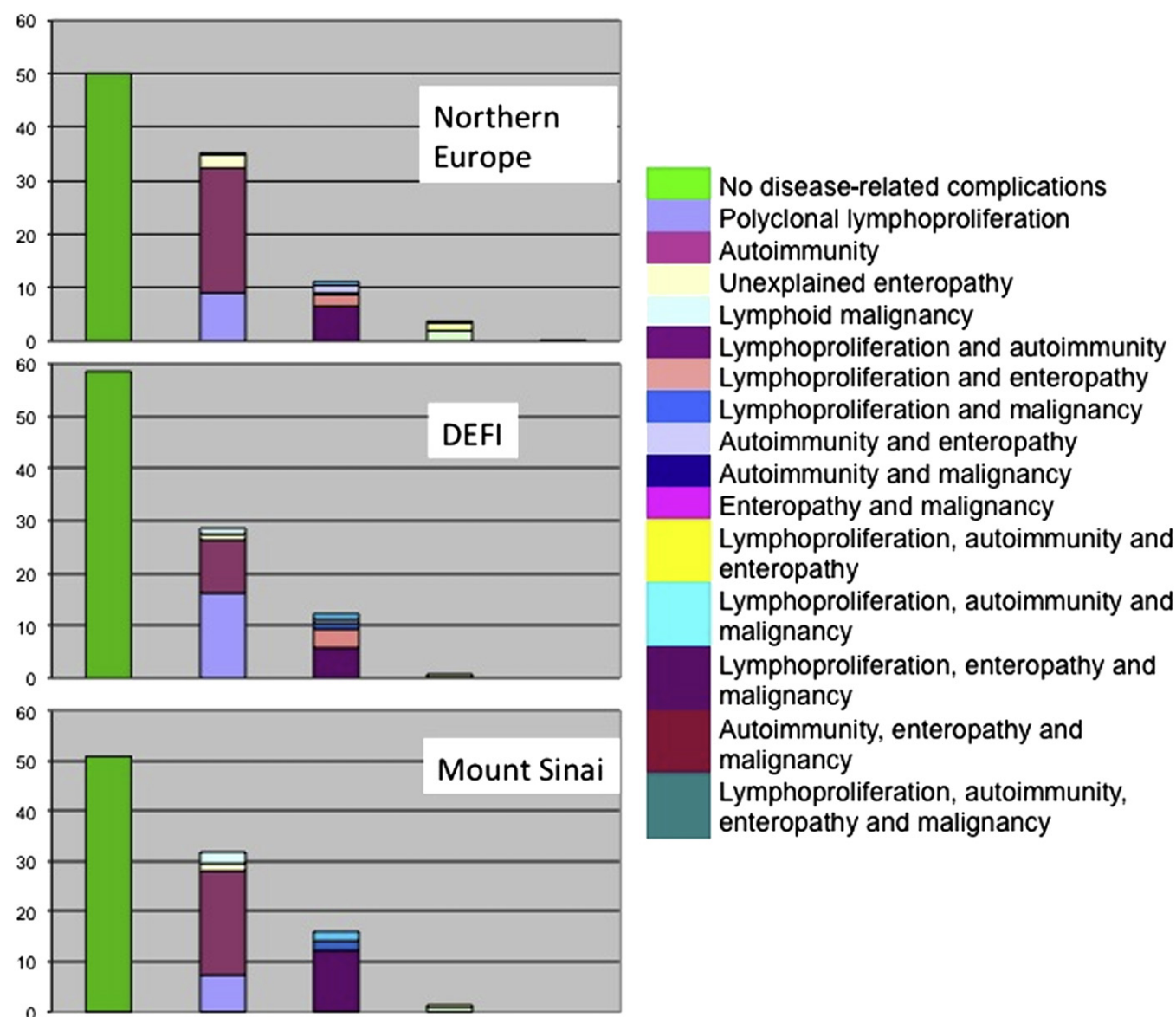


FIG E1. Confirmation of clinical phenotyping using the original criteria.

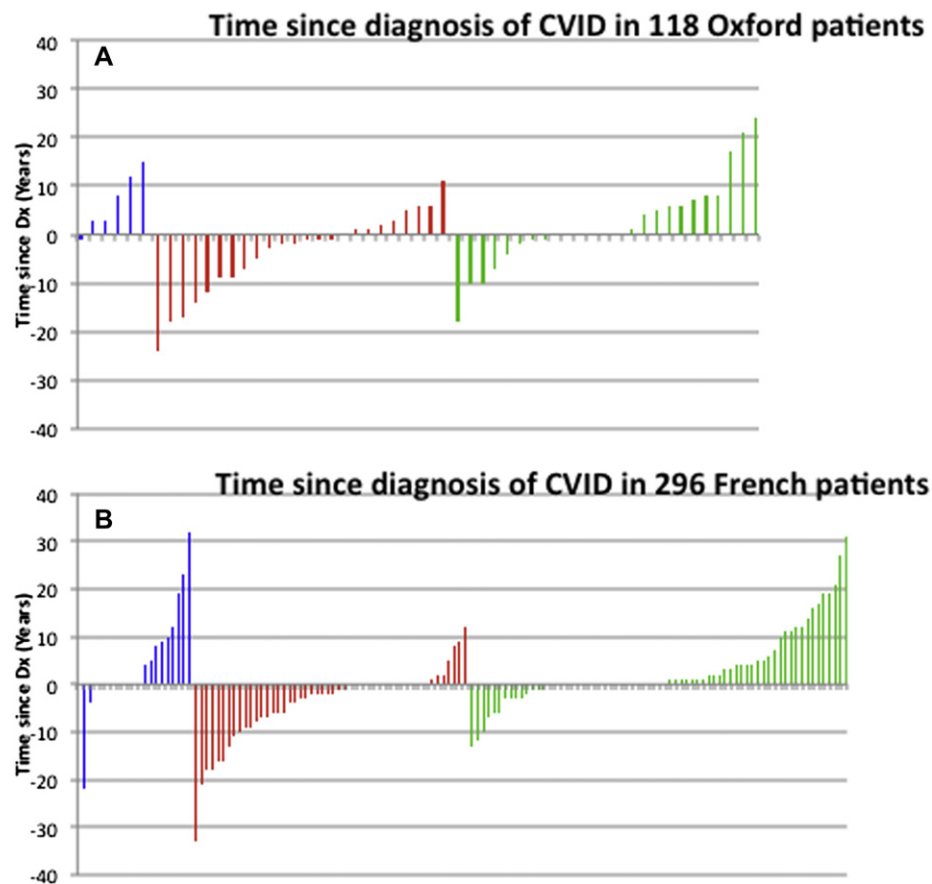


FIG E2. Time interval (years) between the diagnosis of CVID and the onset of complications used for clinical phenotyping in the 2 cohorts: extended Oxford (**A**) and DEFI (**B**). *Dx*, Diagnosis.

TABLE E1. Comparison of previously defined clinical phenotypes with improved clinical phenotypes

Original clinical phenotype criteria	Improved clinical phenotype criteria
Autoimmunity (including organ-specific autoimmune diseases and cytopenias)	Autoimmune cytopenias
Enteropathy (unexplained—excluding infective, autoimmune, and gluten-sensitive enteropathies)	Enteropathy (unexplained—excluding infective, autoimmune, and gluten-sensitive enteropathies)
No disease-related complications (described as “infections only”)	No disease-related complications (described as “infections only”)
Polyclonal lymphoproliferation (including hepatomegaly, persistent unexplained lymphadenopathy, LIP, and noninfective granuloma)	Polyclonal lymphoproliferation (including persistent unexplained lymphadenopathy, LIP, and noninfective granuloma)
Lymphoid malignancies	

TABLE E2. Prognostic indicators for clinical phenotypes (*P* values are shown for significance for survival for each feature)

		Age at diagnosis (n = 739)	Age at onset (n = 703)	Diagnostic delay (n = 695)	Sex (n = 846)	Initial IgG (g/L) (n = 762)	Initial IgA (g/L) (n = 750)	Initial IgM (g/L) (n = 750)	Initial CD number × 10 ⁹ /L (n = 412)	Initial CD8 number (× 10 ⁹ /L) (n = 412)	Initial CD19 number (× 10 ⁹ /L) (n = 525)	Initial CD4/CD8 (n = 702)
Clinical phenotypes	No disease-related complications (n = 552)	.61	.21	.55	.95	.84	.01*	.83	.59	.66	.12	.97
	Cytopenia (n = 138)	.21	.49	.53	.56	.02†	.40	.35	.10	.97	.27	.24
	LP (n = 190)	.85	.24	.65	.63	.09	.02‡	.72	.52	.94	.10	.77
	Enteropathy (n = 52)	.53	.25	.20	.71	.42	.09	.45	.70	.53	.89	.88
Common complications	OSAI (n = 119)									.61		
	Hepatomegaly (n = 60)					.50	.77	.02§	.02 			

Boldface text indicates statistically significant values; n = number of patients in each group.

OSAI, Organ-specific autoimmunity.

*Increase of 180% in the odds of no disease-related complications for each 1 g/L increase in IgA.

†Increase of 14% in the risk of cytopenia for every 1 g/L increase in IgG.

‡Decrease of 69% in the odds of LP for every 1 unit increase in IgA; significance lost when split into granuloma (n = 49), lymphadenopathy (n = 68), and LIP (n = 28), probably because of very low numbers.

§Increase of 50% in the odds of hepatomegaly for each 1 g/L increase in IgM.

||Decrease of 85% in the odds of hepatomegaly for each 1 × 10⁹/L increase in CD4 numbers.

TABLE E3. Clinical phenotypes in 2 age groups

	Proportion with age at diagnosis <16 y (n = 84)	Proportion with age at diagnosis ≥16 y (n = 655)	<i>P</i> value (χ^2 test for homogeneity)
Clinical phenotypes			
No disease-related complications	0.64	0.66	.72
Cytopenia	0.23	0.13	.02
LP	0.24	0.23	.83
Enteropathy	0.06	0.07	.79

Boldface text indicates statistically significant values.

TABLE E4. Evaluation of β -2 microglobulin levels

Phenotype	<i>P</i> value for difference in mean β -2 microglobulin levels with and without phenotype	Unadjusted mean $\pm$ SD of β -2 microglobulin without phenotype	Unadjusted mean $\pm$ SD of β -2 microglobulin with phenotype
LIP	.0002	2.7 $\pm$ 1.6	4.7 $\pm$ 3.2
Splenomegaly	$<10^{-6}$	2.1 $\pm$ 1.2	4.2 $\pm$ 2.6
Bronchiectasis	.71		
Granuloma	.002	2.7 $\pm$ 1.5	4.8 $\pm$ 3.2
Nodular regenerative hyperplasia	.02	3.2 $\pm$ 2.4	4.0 $\pm$ 1.6
Enteropathy	.09		
Cytopenia	.03	3.3 $\pm$ 2.5 (median = 2.7)	3.1 $\pm$ 1.5 (median = 3.0)

TABLE E5. Association of clinical and laboratory findings with survival

	Clinical phenotypes				Common complications			Laboratory parameters			
	Cytopenia	LP	Enteropathy	No disease-related complications	Hepatomegaly	Splenomegaly	Bronchiectasis	Initial CD19 number ($\times 10^9/L$)	Initial IgG (g/L)	Initial IgA (g/L)	Initial IgM (g/L)
Survival	0.003 With cytopenia shorter survival	<0.0001 With LP shorter survival	0.0005 With enteropathy shorter survival	<0.0001 With no disease-related complications longer survival	<0.0001 With hepatomegaly shorter survival			0.02*	0.10	0.59	0.24
Cox regression model						<0.0001 RR = 4.35	0.04 RR = 1.82				

Boldface text indicates statistically significant values.

RR, Relative risk.

*Mean $\pm$ SD for initial CD19 counts $0.26 \pm 0.41 \times 10^9/L$ (median 0.12) in those who survived and mean $\pm$ SD $0.18 \pm 0.27 \times 10^9/L$ (median 0.06) in those who died.