Immunological biomarkers associated with survival in a cohort of Argentinian patients with common variable immunodeficiency

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Background: Common variable immunodeficiency (CVID) is the most common symptomatic syndrome among inborn errors of immunity. Although several aspects of CVID immunopathology have been elucidated, predictive factors for mortality are incompletely defined. A genetic cause can be identified only in approximately 30% of patients.

Objective: We sought to develop a mortality predictive score on the basis of the immunophenotypes and genotypes of patients with CVID.

Methods: Twenty-one patients diagnosed with CVID in Córdoba, Argentina, were analyzed for clinical and laboratory data. Immunophenotyping was done by flow cytometry. CVID-associated mutations were identified by whole-exome sequencing.

Results: Alive (15) and deceased (6) patients were compared. Univariate analysis showed significant differences in $CD4^+$ T cells ($P = .002$), natural killer (NK) cells ($P = .001$), and memory switched B cells ($P = .001$) between groups. Logistic regression analysis showed a negative correlation between $CD4^+$, NK, and memory switched B-cell counts and probability of survival over a 10-year period ($CD4^+$ T cells: odds ratio [OR], 1.01; 95% CI, 1.001-1.020; NK cells: OR, 1.07; 95% CI, 1.02-1.17; and memory switched B cells: OR, 26.23; 95% CI, 2.06-2651.96). Receiver-operating characteristic curve analysis identified a survival cutoff point for each parameter ($CD4^+$ T cells: 546 cells/mL; AUC, 0.87; sensitivity, 60%; specificity, 100%; memory switched B cells: 0.84 cells/mL; AUC, 0.92; sensitivity, 100%; specificity, 85%; and NK cells: 45 cells/mL; AUC, 0.92; sensitivity, 83%; specificity, 100%). Genetic analysis on 14 (9 female and 5 male) patients from the cohort revealed mutations associated with inborn errors of immunity in 6 patients.

Conclusions: A score to predict mortality is proposed on the basis of $CD4^+$ T, NK, and memory switched B-cell counts in patients with CVID. (J Allergy Clin Immunol Global 2024;3:100311.)

Key words: *CVID, score, mortality, outcomes, WES, B-cell subpopulations, T cells*

Common variable immunodeficiency (CVID) is the most common symptomatic syndrome among inborn errors of immunity.[1,2] The precise causes of CVID remain to be elucidated.[3] Clinical manifestations include recurrent and severe infections predominantly of the respiratory and gastrointestinal tracts, autoimmunity, inflammatory diseases, and malignancies, with the latest thought to be a consequence of immune dysregulation.[4-6] Patients classically show low serum IgG and IgA/IgM and reduced antibody response to vaccination in the absence of other causes of hypogammaglobulinemia.[3,7,8] The severity of this disease shows great variability, ranging from just scarce infections throughout life to a severe and fatal disease. The overall prevalence has been estimated to be between 0.5 and 4 cases per 100,000 among White individuals.[4,9] Because there is no CVID-pathognomonic clinical or laboratory findings, the diagnosis relies on consensus criteria.[10-12] Although hypogammaglobulinemia is the common feature in the pathophysiology of CVID, alterations in almost all components of the immune system have been described.[13-17] This clinical and immunological variability strongly suggests that different genetic defects underlie this disorder.[18,19] A genetic cause can be identified only in approximately 30% of patients previously diagnosed with CVID.[20] With the increasing access to whole-exome sequencing (WES) or whole-genome sequencing analysis, several studies have identified monogenic causes in patients with CVID over the last few years. Autosomal gene mutations (*TNFSF12*, *CTLA4*, *PLCG2*, *NFKB1*, *NFKB2*, *PIK3CD*, *PIK3R1*, *VAV1*, *BLK*, *IKZF1*, and *IRF2BP2*), recessive gene mutations (*ICOS*, *CD19*, *CD20*, *CD21*, *CD27*, *CD81*, *IL21*, *IL21R*, *LRBA*, *PRKCD*, and *RAC2*), as well as monoallelic or biallelic mutations (*TNFRSF13B* and *TNFRSF13C*) have been described in genetic analysis.[21] Genetic diagnosis is a potential tool adding important information that may alter the clinical management of patients, giving the opportunity to receive specific treatment.

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Abbreviations used
AUC: Area under the curve
CVID: Common variable immunodeficiency
NK: Natural killer
OR: Odds ratio
PID: Primary immunodeficiency
ROC: Receiver-operating characteristic
WES: Whole-exome sequencing

Treatment of CVID includes life-long immunoglobulin replacement and antimicrobial therapy.[22] This reduces the frequency of bacterial infections, but does not protect against most noninfectious complications, which in turn lead to increased morbidity and mortality compared with the general population.[23-26] Indeed, it has already been shown that the mortality risk is 11 times higher among treated patients with 1 or more noninfectious complication.[9] Interestingly, Resnick et al[9] found low baseline IgG serum levels, decreased peripheral B cells, and increased IgM serum levels as immunological biomarkers associated with reduced survival in patients with CVID. Besides, a retrospective study that enrolled patients with CVID over a 20-year period showed a survival rate of 65% at 6.5 years after diagnosis, with respiratory failure associated with infections being the most frequent cause of death.[27] In addition, Pensieri et al[28] reported that oncoimmunological disorders were another main cause of death in patients with CVID and duodenal villous atrophy.

The fact that CVID is characterized by a broad and heterogeneous clinical spectrum associated with very different outcomes highlights the critical need to identify clinical and/or biochemical predictive parameters for prognosis. Several strategies to classify patients with CVID to predict disease course have been developed.[29] Classifications were based on abnormalities in the B-cell compartment, in which impaired B-cell differentiation and low to absent count of memory switched B cells were associated with different clinical manifestations and worse prognosis.[30-32] Nonetheless, abnormalities in the T-cell compartments have also been reported.[14] Among others, CVID-associated T-cell abnormalities include reduced number of T cells, decreased T-cell proliferation on mitogen and antigen stimulation, defective T-cell signaling, and low count of regulatory T cells.[14,33,34] Patients with CVID with T-cell abnormalities show a more severe disease phenotype, which typically presents with gastrointestinal complications, splenomegaly, granulomas, and lymphomas.[33,34]

Although it has already been found that certain B- and T-cell abnormalities are associated with severe clinical manifestations and disease prognosis, there is still a need to identify novel biochemical and immunological biomarker predictors of outcome and survival. In the present study, we identified immunological parameters that are associated with mortality risk in a cohort of patients with CVID followed over a period of 2 decades. Our data allow us to propose a new scoring system to predict mortality risk in patients with CVID in a cohort from Córdoba, Argentina.

METHODS

Study design, patients, and samples

This prospective longitudinal study enrolled a cohort of 21 patients (13 female and 8 male patients) aged 14 to 52 years, assisted at Hospital Privado Universitario de Cordoba Allergy and Immunology Clinic, Argentina, from 2000 to 2020. CVID was diagnosed according to the criteria of the European Society for Immunodeficiencies, which included the following: (1) hypogammaglobulinemia with IgG levels 2 SD below the mean, (2) impaired vaccine response or absent isohemagglutinins, and (3) exclusion of other causes of hypogammaglobulinemia.[5,12,35] Exclusion criteria were as follows: (1) Agammaglobulinemia, hyper-IgM syndrome, X-linked lymphoproliferative syndrome, combined immunodeficiencies, unstable chromosome syndrome, lymphoma, chronic leukemia, Good syndrome, kidney disease, enteropathies, extensive burns, drug-induced hypogammaglobulinemia (sulfasalazine, glucocorticoids, gold salts, azathioprine, D-penicilamine, antimalarial drugs, methotrexate, cyclophosphamide, chlorambucil, rituximab, and imatinib), congenital cytomegalovirus infection, toxoplasmosis, rubella, neonatal HIV, and EBV infection; (2) younger than 4 years; (3) immunosuppressor therapy; and (4) cancer.

Serum concentrations of total IgG, IgA, and IgM were assessed by standard automated chemiluminescent ELISAs (cobas c 503, Roche Diagnostics, Rotkreuz, Switzerland) before IgG replacement therapy. Delay in diagnosis was defined as the difference between the date of onset of symptoms and the date of established diagnosis. All patients received regular intravenous immunoglobulin replacement therapy and were free of infections when they donated blood samples for the different assays performed.

Ethical approval

The study was carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) standards and the Argentinian legislation for protection of personal data (Law 25326). The experimental protocol was approved by the Ethics Committee and Internal Review Board (Comité Institucional de Evaluación Ética de las Investigaciones en Salud) of Hospital Privado Universitario de Cordoba. Participation in the study was voluntary, and all participants provided a signed written informed consent.

Immunophenotyping

Peripheral blood samples were obtained and collected in sterile heparinized tubes. Red blood cells were eliminated using anti–Corynebacterium kutscheri lysis buffer (Gibco), and cell suspensions of 1×10^7 cells/mL were stained with fluorescent-labeled mAbs specific for human CD3, CD4, CD8, CD56, CD19, CD27, IgM, or IgD (Becton Dickinson Biosciences, San Diego, Calif) to identify total ($CD3^+$) T cells, helper ($CD4^+CD3^+$) and cytotoxic ($CD8^+CD3^+$) T cells, natural killer (NK) ($CD56^+$) cells, total ($CD19^+$) B cells, and B-cell subsets: mature ($CD19^+CD27^+IgM^+IgD^+$), immature ($CD19^+CD27^-$), and memory switched ($CD19^+CD27^+IgM^-IgD^-$) B cells. Cells were acquired on a FACSCanto II cytometer (BD Biosciences, San Jose, Calif) and analyzed using the FlowJo software, version 7.6.2 (Initrogen, Buenos Aires, Argentina). A total of 100,000 cells per sample were analyzed. Dead cells were excluded using LIVE-DEAD fixable (Invitrogen). Proper compensation using Fluorescence Minus One was applied. The lymphocyte cell population was identified using a proper forward versus side scatter gating strategy. To calculate the absolute number of each lymphocyte cell subset, the proportion of cells staining positive was multiplied by the absolute peripheral blood lymphocyte count.

TABLE I. Demographic, clinical, and immunological variables analysis between survivors and deceased patients with CVID

Variable	All (N = 21)	Deceased (n = 6)	Survivors (n = 15)	*P* value
Sex: female/male	13/8	4/2	9/6	.99
Age (y) at diagnosis, mean ± SD	32.82 ± 14.16	40.33 ± 18.22	29.80 ± 12.43	.12
Age (y) at symptoms onset, mean ± SD	28.71 ± 13.45	36.16 ± 17.10	25.73 ± 10.99	.08
Average delay in diagnosis	4.14 ± 3.59	4.16 ± 3.54	4.13 ± 3.73	.75
IgG (mg/dL), mean ± SD	140.0 ± 97.8	97.5 ± 64.6	157.0 ± 105.3	.137
IgA (mg/dL), mean ± SD	5.6 ± 4.6	4.5 ± 4.0	6.1 ± 4.9	.452
IgM (mg/dL), mean ± SD	34.7 ± 41.2	23.0 ± 33.5	39.7 ± 44.2	.372
IgE (UI/mL), mean ± SD	1.5 ± 0.9	1.5 ± 0.5	1.5 ± 1.0	.937
Autoimmune cytopenia (%)	9 of 21 (42)	4 of 6 (67)	5 of 15 (33)	.33
Splenomegaly (%)	11 of 21 (52)	5 of 6 (83)	6 of 15 (40)	.14
Enteropathy (%)	7 of 21 (33)	4 of 6 (67)	3 of 15 (20)	.11
Bronchiectasis (%)	11 of 21 (52)	4 of 6 (67)	7 of 15 (47)	.63
Cancer	3 of 21 (14)	1 of 6 (17)	2 of 15 (13)	1
Infections-only	9 of 21 (43)	1 of 6 (17)	8 of 15 (53)	.63
Lymphocytes (cells/mL), mean ± SD	1711 ± 1124	1132.2 ± 809.1	1943 ± 1171	.093
CD3 (cells/mL), mean ± SD	1274 ± 655	981 ± 671	1391 ± 633	.232
CD4 (cells/mL), mean ± SD	598.62 ± 334.12	296.00 ± 194.88	719.67 ± 301.62	**.002**
CD8 (cells/mL), mean ± SD	618.90 ± 391.85	636.00 ± 469.71	612.07 ± 374.67	.914
CD19 (cells/mL), mean ± SD	184 ± 133	108 ± 121	217 ± 128	.097
$CD19^+CD27^+IgD^-/IgM^-$ (memory switched B cells), median (IQR)	1.32 (0.36; 4.70)	0.31 (0.00; 0.36)	2.75 (1.18; 7.00)	**<.001**
$CD19^+/CD27^+$ (cells/mL), mean ± SD	46.03 ± 42.60	30.83 ± 39.76	52.4 ± 43.50	.301
NK (cells/mL), mean ± SD	88.55 ± 57.14	38.17 ± 26.97	110.14 ± 53.10	**<.001**

Boldface indicates statistical significance at $P < .05$.
IQR, Interquartile range.

Whole-exome sequencing

The genetic diagnosis was made by WES, and the detected variant was confirmed by Sanger sequencing. Briefly, genomic DNA was extracted from peripheral blood samples and 1 μg of DNA was used for exome capture using the IDT XGen exome target design or Agilent SureSelect Human All Exon. Generated libraries were sequenced using 75-bp paired-end sequencing on an Illumina NovaSeq-6000 and BGISeq-500 platform. Captured fragments were sequenced to achieve a minimum of 85% of the target bases at 20× or greater coverage. Analysis of WES data was performed using the Variant Explorer pipeline to narrow down potential candidate variants. Raw data were processed, filtered, and analyzed according to the Variant Explorer pipeline recommendations. Candidate genes were further evaluated by our research team. Sanger sequencing was performed to confirm the mutation identified by WES. Briefly, genomic DNA was amplified by a PCR, and amplimers were sequenced using the Big Dye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems/Life Technologies, Darmstadt, Germany) on an Applied Biosystems 3130 Genetic Analyzer.

Targeted gene screening

WES results were screened for genes associated with immunodeficiency, collated and reported by the International Union of Immunological Sciences Committee on Inborn Errors of Immunity.

Statistical analysis

Continuous normal and nonnormal distributed variables were reported as mean ± SD and median (interquartile range), respectively, with differences between groups assessed using the Student *t* test and the Wilcoxon rank-sum test (Mann-Whitney *U* test). Dichotomous variables were presented as absolute numbers (n) and relative frequencies (%), with differences between groups assessed using the Fisher exact test. Weighted ordered logistic regression analysis was performed in the construction of the score to identify the variables with an independent predicting capacity and to obtain the variables' scoring weights. Receiver-operating characteristic (ROC) curve analysis was performed to determine the cutoff value predictor of mortality for variables considering the best combination between sensitivity and specificity, with analysis of the area under the curve (AUC). Kaplan-Meier tests were performed for survival analysis over a 10-year period, and the log-rank trend test was performed for analysis of differences between groups. Sensitivity, specificity, and positive and negative predictive values were calculated and expressed as percentages (%) with 95% CIs. A *P* value of less than .05 was considered statistically significant. Analyses were conducted using the R software, version 4.0.2 (R Project for Statistical Computing [worldwide collaborative project]).

RESULTS

Demographic and immunological markers associated with fatal outcome

The patient cohort under study included 21 patients (13 female and 8 male patients) with confirmed diagnosis of CVID,[27,35] assisted at Hospital Privado Universitario de Cordoba Allergy and Immunology Clinic from 2001 to 2020. Patient mean age was 28.71 ± 13.45 years at symptoms onset and 32.82 ± 14.16 years at diagnosis, indicating a diagnostic delay of 4.14 ± 3.59 years (Table I). All patients showed decreased serum IgG, IgM, IgA, and IgE concentrations (Table I), which remained constant over time. Throughout the analyzed period, 42% of patients developed autoimmune cytopenia including anemia or thrombocytopenia, 52% showed splenomegaly, 33% developed chronic diarrhea, 52% had bronchiectasis, and 14% developed cancer. Fifteen patients (71%) had a history of severe or recurrent infections, predominantly of the respiratory tract, with the identified etiologic

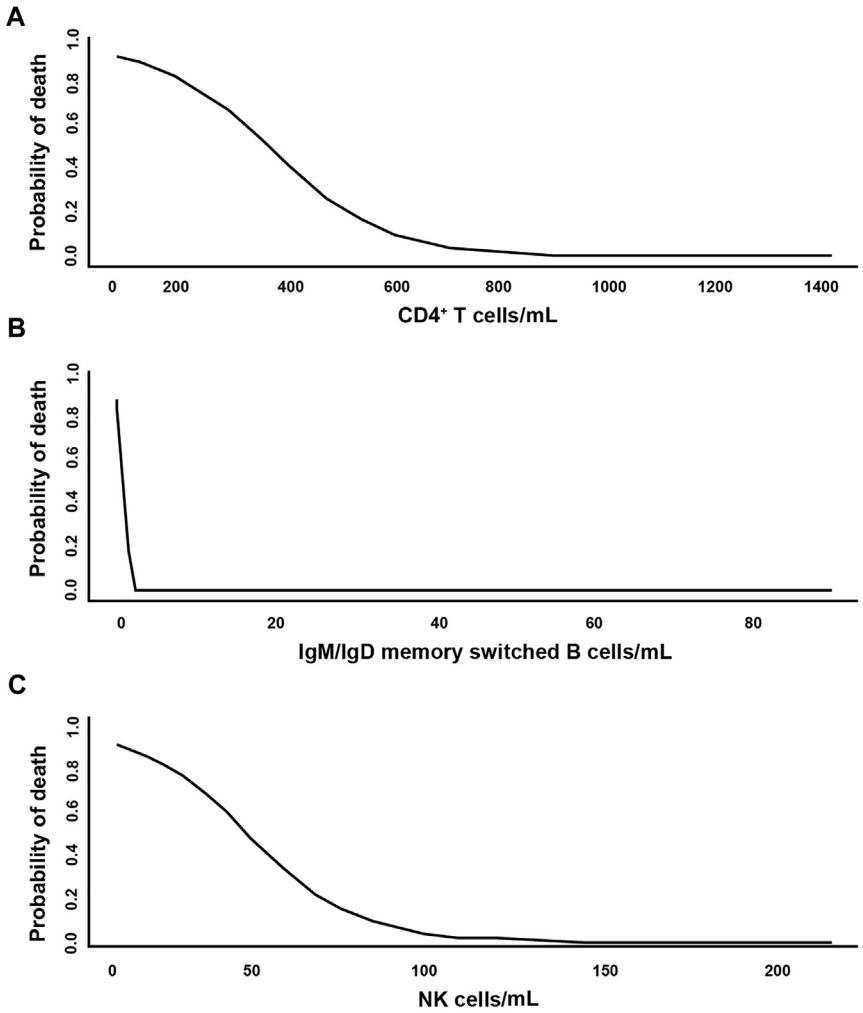


FIG 1. Probability of death according to circulating immune subset counts. **A,** Probability of death according to circulating $CD4^+$ T-cell counts. **B,** Probability of death according to circulating IgM/IgD memory switched B-cell counts. **C,** Probability of death according to circulating NK-cell counts. The outcome studied is the cumulative incidence of death.

pathogens being *Streptococcus pneumoniae*, *Moraxella catharralis*, *Escherichia coli*, *Haemophilus influenzae*, *Influenza virus*, and *Candida albicans*. Of the 21 patients, 9 (43%) developed severe recurrent infections without other clinical manifestations. When analyzing patient survival, 6 patients (4 female and 2 male patients) of the 21 patients died during the study time period, indicating a mortality rate of 28%. The median age at death was 48.00 ± 16.29 years, and the predominant causes of death were severe infections, unclassified enteropathy, and malignancy.

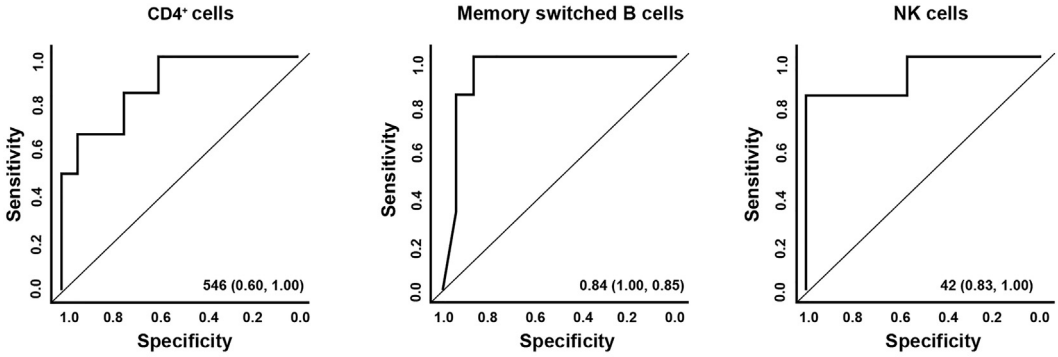
Univariate analysis was used to compare different clinical and biochemical parameters between dead or alive patients during the study (Table I). No significant differences in any of the demographic variables analyzed (ie, sex, age at diagnosis, age at symptoms onset, and average delay for diagnosis), the frequency of clinical findings (ie, autoimmune cytopenia, splenomegaly, enteropathy, bronchiectasis, cancer, and infections), or serum level of immunoglobulin subtypes (IgG, IgM, IgA, and IgE) were found between deceased and alive patients with CVID ($P > .05$). The number of total lymphocytes, T and B cells, and their subpopulations were also analyzed. There were no significant differences in lymphocytes ($P = .093$), $CD3^+$ T cells ($P = .232$), $CD8^+$ T cells ($P = .914$), and $CD19^+$ B cells ($P = .097$) between deceased and alive patients with CVID (Table I). However, the absolute number of $CD4^+$ T cells was significantly reduced in patients who died during the study in comparison with survivors ($P < .002$). Within the B-cell compartment, $CD19^+CD27^+IgM^-IgD^-$ memory switched B cells were strikingly reduced in deceased patients with CVID when compared with survivors ($P < .001$), whereas no significant differences were found when analyzing total $CD19^+CD27^+$ B cells ($P < .301$). The number of circulating NK cells in patients who died during the study was also significantly lower than in survivors ($P < .001$).

In parallel, association among variables under study was assessed by logistic regression analysis. Death was defined as the dependent variable and the absolute number of $CD4^+$ T cells, NK cells, and memory switched B cells as the independent variables. As shown in Fig 1 (see also Table II), there was significant negative correlation between the absolute number of circulating

TABLE II. Logistic regression analysis showing the association between significant immunological predictors and death outcome in patients with CVID

Variable	Coefficient	SE	OR	95% CI	*P* value
$CD4^+$ T cells	−0.01	0.00	1.01	1.00-1.02	**.034**
NK cells	−0.07	0.03	1.07	1.02-1.17	.054
Memory switched B cells	−3.27	1.75	26.23	2.06-2651.96	.062

Boldface indicates statistical significance at $P < .05$.



Variable	Cutoff value	Sensitivity	Specificity	AUC
$CD4^+$ T cells	546	60	100	0.87 (0.71, 1.00)
Memory switched B cells	0.84	100	85	0.92 (0.80, 100)
NK cells	42	83	100	0.92 (0.78, 100)

FIG 2. Cutoff values for immunological variables that might predict CVID survival. ROC curves for differential $CD4^+$ cells, memory switched B cells, and NK cells are shown. The AUC is 0.87 for $CD4^+$ T cells and 0.92 for memory switched B cells and NK cells.

$CD4^+$ T cells, NK cells, and memory switched B cells and the probability of survival ($CD4^+$ T cells: odds ratio [OR], 1.01; 95% CI, 1.001-1.020; NK cells: OR, 1.07; 95% CI, 1.02-1.17; and memory switched B cells: OR, 26.23; 95% CI, 2.06-2651.96). These results indicate that a low number of peripheral blood circulating memory switched B cells, NK cells, and $CD4^+$ T cells is a risk factor associated with worse disease outcome and decreased survival in patients with CVID.

Development of a new immunological score as a predictive tool of worse CVID disease outcome

Because peripheral blood cell counts of $CD4^+$ T cells, NK cells, and memory switched B cells were biomarkers significantly associated with worse disease outcome and death, ROC curve analysis was performed to set cutoff points that might help predict survival. As shown in Fig 2, the calculated cutoff value for $CD4^+$ T-cell count was 546 cells/mL (AUC, 0.87; sensitivity, 60%; and specificity, 100%), for memory switched B cells, 0.84 cells/mL (AUC, 0.92; sensitivity, 100%; and specificity, 85%), and for NK cells, 45 cells/mL (AUC, 0.92; sensitivity, 83%; and specificity, 100%).

To build a disease outcome predictive immunological score, we assigned different points to each of the 3 aforementioned biomarkers using their calculated cutoff values. Taking into account the significance of the association between mortality and the biomarkers assayed, a score of 0 to 4 was built; a total of 2 points for memory switched B-cell count and 1 point for either NK or $CD4^+$ T-cell count were assigned when patient values were less than the calculated cutoff values, respectively (Fig 3, *A*). ROC curve analysis gave an optimal cutoff score point indicating that values greater than 2.5 could serve as predictive of high mortality risk in patients with CVID with a sensitivity and specificity of 83% and 100%, respectively (Fig 3, *B*). Moreover, the positive predictive value was 1.00 (95% CI, 0.48-1.00), whereas the negative predictive value was 0.94 (95% CI, 0.70-1.00).

We calculated the score for every patient with CVID under study. As provided in Table III, 11 patients presented $CD4^+$ T-cell counts lower than 546 cells/mL, 5 patients presented memory switched B-cell counts lower than 1 cell/mL, and 6 patients presented NK-cell counts lower than 45 cells/mL. Thus, 5 of the 21 patients under study had a disease outcome predictive score greater than or equal to 2.5, indicating they were at high risk of worse disease outcome or death. These 5 patients were included in the 6 who died in the study. Interestingly, 9 of the 21 patients exhibited the "infections-only" phenotype (Table III). Consistent with the previous association of this phenotype with reduced mortality,[18] all of the infections-only patients demonstrated markedly low scores (mean score, 0.22 vs 2.00 for the infections-only patients vs the other patients; $P = .0045$).

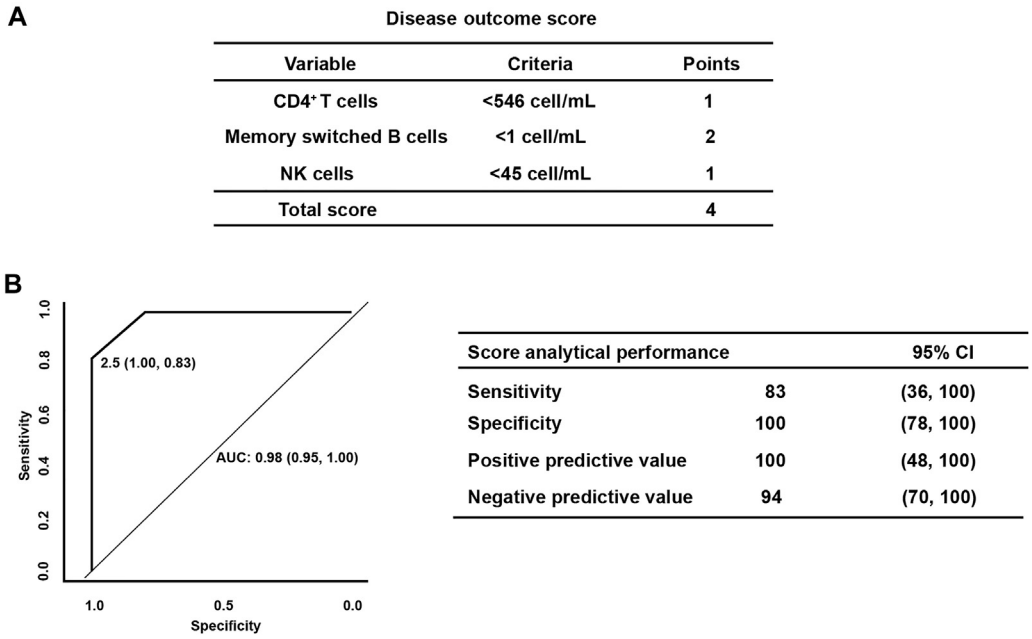


FIG 3. Construction of a disease outcome and survival score. **A,** Points assigned for $CD4^+$ T cells, memory switched B cells, and NK cells when greater than calculated cutoff values. **B,** ROC curve showing a cutoff score point of 2.5, predictive of high mortality risk in patients with CVID.

TABLE III. Disease outcome score calculated for the CVID population under study

Patient	$CD4^+$ T cells/mL	Memory switched B cells/mL	NK cells/mL	Infections-only	Score
1	648	26	147	No	0
2	81	<1	36	No	**4**
3	123	<1	12	No	**4**
4	950	3	71	Yes	0
5	534	<1	158	No	3
6	479	6	76	No	1
7	486	24	42	No	1
8	353	<1	26	No	**4**
9	404	3	54	Yes	1
10	187	<1	24	No	**4**
11	937	7	146	Yes	0
12	289	4	117	No	1
13	546	12	89	Yes	0
14	709	11	59	Yes	0
15	902	7	47	No	0
16	711	90	216	Yes	0
17	1113	9	58	Yes	0
18	1431	26	0	No	1
19	484	5	112	Yes	1
20	539	3	182	No	1
21	604	7	99	Yes	0

Boldface values denote score numbers greater than or equal to score 2.5.

Kaplan-Meier survival analysis confirmed our observations and showed a significantly reduced survival probability in those patients having $CD4^+$ T-cell counts less than the calculated cutoff of 546 cells/mL, with a 10-year survival of 50% versus 100% for those having more than 546 cells/mL ($P = .03$; Fig 4). Similarly, patients having NK-cell counts less than the cutoff of 45 cells/mL showed a 10-year survival probability of 20% versus 92% for those having more than 45 cells/mL ($P = .007$; Fig 4). Finally, patients having memory switched B-cell counts less than the cutoff of 1 cell/mL exhibited a 10-year survival of 33% versus 87.5% for those having more than 1 cell/mL ($P = .004$; Fig 4).

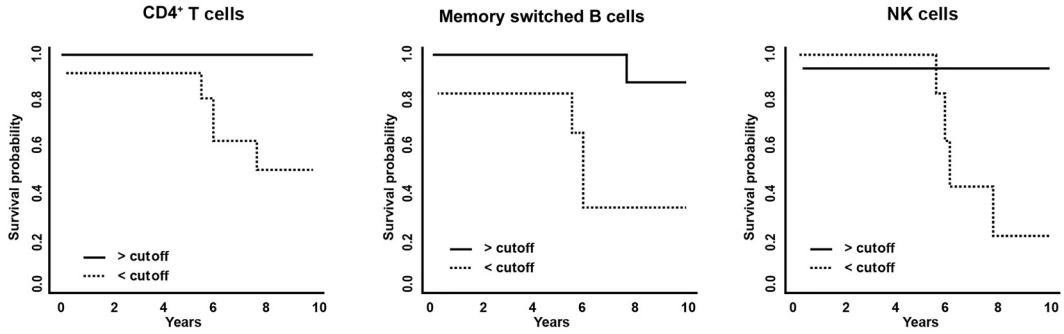
Altogether, our results indicate that reduced numbers of circulating $CD4^+$ T cells, NK cells, and memory switched B cells are important predictors for worse outcome/reduced survival in patients with CVID. A score constructed with the combination of these 3 immunological biomarkers would be useful to predict disease outcome in patients with CVID, helping to improve patient care and quality of life.

Whole-exome sequencing

We analyzed genetic mutations using WES in 14 patients (9 female and 5 male patients) and their families when available for mutations known to be associated with CVID (Table IV). We identified 6 mutations considered pathogenic in 6 patients (42%). Clinically, 5 patients had the infections-only phenotype[18] and 1 had hemolytic anemia. There was no consanguinity known by patients, and only 1 had a history of IgA deficiency in her family. We identified mutations in APRIL, *IKBKB*, TCF3, TRAC, TTC7A, and IL2RG. The mutations did not specifically segregate with patients with worse disease prognosis. Indeed, the patient carrying the TCF3 mutation showed CD4 values lower than the cutoff value (score = 1), with a 100% survival among patients with identified mutations.

DISCUSSION

In the present study, we described clinical characteristics and disease outcome in a cohort of patients with CVID and developed a predictive score able to estimate the risk of mortality in these patients using immunological biomarkers that significantly



10-year survival (log-rank trend test)			
Variable	Survival (%) > cutoff	Survival (%) < cutoff	*P*
$CD4^+$ T cells	100 (99,100)	50 (19,76)	0.030
Memory switched B cells	87 (38, 98)	33 (5, 67)	0.004
NK cells	92 (59, 98)	20 (0, 59)	0.007

FIG 4. CVID probability survival analysis. Kaplan-Meier curves showing 10-year survival probability in patients with CVID with $CD4^+$ T cells, memory switched B cells, and NK cells when greater than or less than the cutoff values.

TABLE IV. Gene mutations, laboratory parameters, and clinical features

Gene found	Inheritance	Clinical features	$CD4^+$ T cells/mL	Memory switched B cells/mL	NK cells/mL	Score	Alive
TNFSF12/13 (APRIL)	Dominant (X-linked)	Hemolytic anemia	709	11	59	0	Yes
IKBKB	Dominant	Pneumonia sinusitis	711	90	216	0	Yes
TCF3 (AGM8)	Dominant	Pneumonia sinusitis	484	5	112	1	Yes
TRAC (ID7)	Recessive (mutation + CNV)	Pneumonia sinusitis	835	6	125	0	Yes
TTC7A	Compound heterozygous	Pneumonia sinusitis	775	1	246	0	Yes
IL-2R (IL-21R)	X-linked	Pneumonia sinusitis	950	3	71	0	Yes

APRIL, A proliferation-inducing ligand; *CNV*, copy number variation.

correlated with worse disease outcome and low survival probability. Specifically, our results show that 3 immunological parameters—the absolute counts of circulating $CD4^+$ T cells, memory switched B cells, and NK cells—are associated with mortality risk. When combined, these 3 biomarkers resulted in a score that could reliably predict patient survival, which is of utmost importance for patients, families, and clinicians.

The development of a scoring system to reliably predict disease survival in patients with CVID would be clinically useful, because it would provide physicians with a tool to predict patients at risk of an earlier death. Although different immunological and disease parameters, including low baseline IgG, high IgM, and noninfectious complications, have previously been associated with higher mortality, no predictive score for mortality has been established so far. In that regard, the scoring system described herein is unique in that no other tool is currently available to predict mortality in CVID, rendering it a particularly useful tool in directing patient care. Individual analyses of $CD4^+$ T cells, memory switched B cells, and NK cells have previously been associated with CVID outcome.[4,9,30,32,36] However, little is known in terms of their potential as immunological mortality predictors. Reduction in the number of T cells and their function has been demonstrated as a central event in the pathophysiology of CVID. Patients from our cohort with worse disease outcome who died during the study time period showed significantly reduced circulating $CD4^+$ T cells compared with survivors. Logistic regression analysis revealed that patients with $CD4^+$ T-cell counts less than the cutoff value determined by ROC curve analysis had significantly lower survival probability. These data suggest that $CD4^+$ T-cell count would be a reliable biomarker to determine patient survival. The relevance of immunophenotyping B cells to classify patients with CVID has been previously shown, demonstrating a correlation between memory switched B cells and clinical manifestations and clinical complications, but no reported study has analyzed these cells as biomarkers of mortality risk in patients with CVID. One of the strengths of this work is that we analyzed memory switched B cells in a cohort of patients with CVID along a 20-year period. Our results revealed significant differences in peripheral blood cell counts of these cells between patients who remained alive and those who finally died. The survival probability at 10 years was significantly lower in patients with memory switched B-cell counts less than the determined cutoff value. These findings place memory switched B cells as a reliable prognostic biomarker for mortality in patients with CVID. Low number of NK cells has also been associated with infectious and noninfectious complications in CVID by different authors. Our results are in agreement with

the reported evidence and altogether suggest circulating NK cells as a potential predictive biomarker of disease outcome and survival in patients diagnosed with CVID.[37] After individual cell analysis ($CD4^+$ T cells, memory switched B cells, and NK cells), we consider that combining them in a prognostic disease score empowers each biomarker performance, resulting in a very helpful clinical tool for patient care. We then propose the use of a mortality prognostic score on the basis of 3 immunological biomarkers capable of being easily implemented in health care institutions of most countries worldwide. Although our results are consistent, additional studies analyzing bigger cohorts of patients are needed to validate our findings.

As part of this study, WES searching for mutations previously associated with primary immunodeficiencies (PIDs) using a targeted bioinformatics analysis was performed. We identified 6 genes associated with PIDs in 6 patients, which represents 42% of studied cases. This percentage is similar to others reported previously. Of the 6 identified gene defects, 3, including *IKBKB*, were associated with autosomal-dominant inheritance, whereas the other 3 were autosomal- or X-linked recessive (*TTC7A* and *IL2RG*).[19] Among the mutations we found in this cohort, 2 were in genes associated with combined immunodeficiencies (*IL2RG* and *IKBKB*)[38] and 1 in *TCF3*,[39,40] mutations in which cause a non–Bruton tyrosine kinase–associated hypogammaglobulinemia. We did not get additional information for the score with the genetic mutations found. An important clinical feature of this cohort is that they were all adults, with a median age of 32 years. Although it contrasts with many PID studies done in children, there are some other adult cohorts described before with a similar median age, showing that genetic studies at this age might be of clinical relevance because it can be found as a disease-associated gene defect in up to almost half of the patients.

A limitation of this study is the small number of patients analyzed, which also limits the scope of genetic defects identified in this cohort and restricts the association of the latter with mortality outcomes. Future studies with a larger number of patients would be required to replicate our results and validate the utility of the mortality predictive score in different clinical settings. Larger studies may also clarify the relationship, if any, between genetic variants associated with patients with CVID and survival outcomes.

Our study lays the groundwork for future efforts to elucidate factors predicting survival in CVID and which are amenable to intervention for the benefit of improved patient care.

DISCLOSURE STATEMENT

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Clinical implications: Immunological features have been described as predictors of clinical outcomes in patients with CVID. A new mortality score of CVID has been proposed on the basis of common immunological markers that might help clinicians in the management of patients diagnosed with CVID.

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