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Brief Communication

Evaluating laboratory criteria for combined immunodeficiency in adult patients diagnosed with common variable immunodeficiency

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ABSTRACT

Some patients diagnosed with common variable immunodeficiency (CVID) actually suffer from combined immunodeficiency (CID) and therefore may require a different, CID-adapted treatment. Several CD4 T-cell-based criteria have been proposed in the past to identify patients with CID within the cohort of adult CVID patients. In this monocentric study, we used retrospective immunological and clinical data of 238 CVID patients to compare four different proposals of how to define CID among CVID patients. We demonstrate that none of the current definitions sufficiently separates CID from CVID patients and that the relative reduction of naïve CD4 T cells < 10% has the highest sensitivity of all tested markers for patients with clinical complications often associated with CID. Thus, a very low percentage of naïve CD4 T cells in any adult CVID patient should raise suspicion, but is not sufficient to define CID among CVID patients.

1. Introduction

Common variable immunodeficiency (CVID) defined according to the ESID/PAGID criteria [1] comprises diverse clinical and immunological phenotypes associated with varied disease course and prognosis [2]. Especially among CVID patients with manifestations of immune dysregulation (complex CVID) features of a cellular immunodeficiency have been observed and these patients may actually suffer from some form of combined immunodeficiency (CID) and potentially require specific disease management beyond immunoglobulin replacement therapy [3–5]. However, the differentiation of patients with clinically relevant CID from those with CVID remains a challenge. In order to define patients with relevant T-cell deficiency, the French DEFI study group had originally proposed the occurrence of opportunistic infections (OI) or a CD4 T-cell count < 200/μl as criteria for 'late-onset combined immune deficiency' in 2009 (DEFI2009-LOCID) [3]. In 2014, the ESID registry revised the CVID diagnostic criteria by the exclusion of CID patients defined by fulfilling at least two of the following criteria: CD4 T-cell count < 200/μl, naive CD4 T cells < 10% or absent T-cell proliferation (ESID2014-CID) [6]. Recently, the DEFI study group modified the LOCID definition, which now classifies patients with OI or a naïve CD4 T-cell count < 20/μl as LOCID (DEFI2015-LOCID) [4]. Here, we compared these three definitions with a fourth alternative (Freiburg-CID) defined by OI or naïve CD4 T

Abbreviations: CID, combined immunodeficiency; CVID, Common variable immunodeficiency; DRC, disease related complication; ESID, European Society for Immune Deficiency; LOCID, late-onset combined immunodeficiency; OI, opportunistic infection; SCID, severe combined immunodeficiency; WES, whole exome sequencing

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Table 1
Patient characteristics of the Freiburg CVID cohort and its CID subgroups.

	all CVID patients		DEFI2009-LOCID			DEFI2015-LOCID			ESID2014-CID			Freiburg-CID			non-CID CVID		
	n/m	IQR/%	n/m	IQR/%	Sn (%)	n/m	IQR/%	Sn (%)	n/m	IQR/%	Sn (%)	n/m	IQR/%	Sn (%)	n/m	IQR/%	Sn (%)
Population (n)	238		24	10		33	14		11	5		74	31		159	67	
Age (y)																	
at diagnosis	34	23–43	28	22–41		32	23–42		26	24–42		36	27–45		32	21–42	
at first symptoms	25	12–35	19	15–29		22	17–35		20	17–30		31	17–39		22	9–33	
at inclusion	45	36–53	48	33–57		44	33–52		51	38–60		49	39–53		45	35–51	
Male gender	118	50	10	42		13	39		5	45		29	39		85	53	
Consanguinity	5	2	1	4		1	3		0	0		1	1		4	3	
Familial case	28	12	2	8		2	6		1	9		8	11		20	13	
Clinical features																	
Infection only[a]	128	54	5	21	4	4	12	3	2	18	2	11	15	9	115	72	90
DRC[b]	109	46	19	79	17	29	88	27	9	82	8	62	84	57	44	28	40
Granuloma	49	21	7	29	14	13	39	27	3	27	6	29	39	59	19	12	39
Autoimmune cytopenia	72	30	15	63	21	23	70	32	8	73	11	47	64	65	23	14	32
Enteropathy	38	16	8	33	21	10	30	26	3	27	8	22	30	58	14	9	37
Lymphoma	10	4	3	13	30	4	12	40	1	9	10	8	11	80	1	1	10
OI[c]	8	3	8	33		8	24		0	0		8	11		na		
OI-only[d]			6	25		7	21		na			5	7		na		
Deaths	17	7	5	21	29	7	21	41	2	18	12	12	16	71	4	3	24

CVID, common variable immunodeficiency; CID, combined immunodeficiency; LOCID, late-onset CID; DEFI2009-LOCID, opportunistic infections (OI) or CD4 T-cell count < 200/µl; DEFI2015-LOCID, OI or naïve CD4 T-cell count < 20/µl; ESID2014-CID, CD4 T-cell count < 200/µl and naive CD4 T cells < 10%; Freiburg-CID, OI or naive CD4 T cells < 10%; DRC, disease related complications; OI, opportunistic infections; m, median; n, number; IQR, interquartile range; Sn, sensitivity.

[a] No granuloma, no autoimmune cytopenia, no enteropathy and no lymphoma.

[b] Granuloma, autoimmune cytopenia, or enteropathy.

[c] All patients with history of opportunistic infections.

[d] Patients with history of opportunistic infections but normal CD4 T-cell-, naïve CD4 T-cell counts or naïve CD4 T-cell percentage according to the respective CID criteria.

cells < 10% in regard to their clinical and immunological validity to identify patients with clinically relevant combined immunodeficiency among adult CVID cohorts.

2. Results and discussion

Table 1 and Table S1 contain retrospective immunological and clinical data of 238 adult CVID patients classified according to the four different schemes (see methods section for in- and exclusion criteria and introduction for classification criteria). 109 (46%) of all CVID patients suffered from disease-related complications (DRCs: granuloma, autoimmune cytopenia or enteropathy) and ten (4%) developed lymphoma. During the observation period 17 patients (7%) died, 12 of these due to DRCs or lymphoma, confirming the worse prognosis of patients with complications [7].

The median age at diagnosis and at first symptoms did not differ significantly between the four “CID” groups. All four “CID” groups comprised slightly fewer male patients (39–45%) when compared to the control group (53%). The prevalence of DRCs (79–88%) or lymphoma (9–13%) was similarly enriched within all four groups, when compared to the total CVID cohort (46% and 4%, respectively) or all non-CID CVID patients (28% and 1%, respectively). Also, mortality was higher in all defined CID groups (16–21%) than among all (7%) or all non-CID CVID patients (3%). The main difference between the four CID definitions was the number of patients fulfilling the respective criteria. Only very few fulfilled the ESID2014-CID criteria, while the Freiburg-CID comprised the largest group (Table 1). Thereby, sensitivity for DRCs, lymphoma, or death, was higher for the Freiburg-CID definition (57%, 80% and 71%, respectively) compared to all other definitions (Table 1). Thus, while in concordance with previous reports [3,4,8] low (naïve) CD4 T cells are associated with complications and a poor prognosis in CVID (Fig. S1), our data indicate that a low proportion of < 10% is a more sensitive criterion rather than absolute numbers of < 20/µl naïve CD4 T cells. ROC curve analyses show however, that both relative and absolute naïve CD4 T cell numbers can separate patients with complications from infection-only patients to a similar degree (Fig. S1C). To reach equivalent sensitivity and specificity as with the Freiburg-CID criteria the cut-off for absolute naïve CD4 T cell numbers would need to be increased to about 50/µl.

When comparing the immunological overlap of the different CID criteria, all DEFI2015-LOCID patients fulfilled also the Freiburg-CID criteria, but not all patients with low CD4 counts had also low naïve CD4 T cells and vice versa (Fig. 1). Analysis of patients with consecutive data who developed both low naïve CD4 T-cell proportions and low CD4 T-cell counts during the observed period showed that seven out of ten patients had dropped naïve CD4 T cells below 10% before their absolute CD4 T-cell numbers dropped (delay 1–14 years). In two patients both criteria were fulfilled within the same year, while only one patient dropped CD4 T-cell counts before the relative loss of naïve CD4 T cells (Fig. S2A).

Of the patients presenting with only one criterion within the

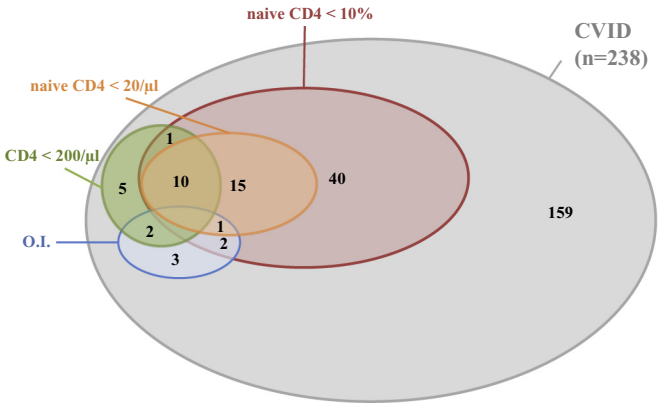

Fig. 1. Distribution of CVID patients according to the different CID criteria. Number and overlap of patients with CD4 T cells < 200/µl (green circle), naïve CD4 T cells < 20/µl (orange circle), naïve CD4 T cells < 10% (red circle), or opportunistic infections (blue circle) among 238 patients with CVID diagnosis. *CVID*, Common variable immunodeficiency; *O.I.*, opportunistic infection.

observation time, 36 patients had < 10% naïve CD4 cells for 1 to 16 years without a drop of CD4 T cells below 200/μl, but four patients had CD4 counts below 200/μl for at least 1 to 5 years without naïve CD4 T cells below 10% (Fig. S2B). Thus, more frequently the relative drop of naïve CD4 cells precedes the absolute loss of CD4 T cells although both features can occur independently. The patients' age at which their cells fulfilled either criterion did not differ (Fig. S2C).

Similar to observations in the French cohort [4], all CID groups had lower B-cell numbers and similarly altered distribution of subpopulations (Table S1, Fig. S3), when compared to the non-CID CVID patients. B-lymphopenia was more pronounced in groups with low absolute CD4 T cells (DEFI-LOCID, 20–32/μl and ESID2014-CID, 11/μl) as compared to Freiburg-CID patients (56/μl), suggesting an underlying panlymphopenia. The proportion of $CD21^{low}$ B cells was significantly elevated only in groups classified on the basis of low naïve CD4 cells when compared to non-CID CVID patients (Table S1, Fig. S3B), as previously reported [5].

Additional characteristic abnormalities that are associated with atypical SCID [9] as expanded TCRγδ-T cells (> 20%) or oligoclonal CD4-TCR Vβ expansion were rare events (0/101 and 4/48 analyzed patients, respectively). All four patients with a skewed CD4 repertoire were found in the Freiburg-CID and three in the DEFI2015-LOCID group (Table S1).

Three patients carried disease-causing homozygous mutations in CID-related genes (table 1 in [10]) (2× ICOS, 1× RAG1). T-cell phenotyping of the RAG1-deficient patient fulfilled DEFI2015-LOCID and Freiburg-CID criteria, one of the two ICOS-patients classified as DEFI2009-LOCID. Whole exome sequencing (WES) of additional 86 patients revealed no additional disease-causing variants in CID-related genes.

Disease-causing mutations in genes associated with antibody deficiency or immune dysregulation (tables 3 and 4 in [10]) were known in eight patients and WES revealed such mutations in another 20 patients, who did not specifically cluster into CID groups (Table S2). These included mutations in *NFKB1*, *NFKB2*, *CTLA4*, *LRBA IKZF1, BACH2* all of which are potentially associated with DRCs and altered cellular immunity. None of them, however, was classified as CID CVID with the exception of one LRBA patient who was classified as LOCID according to both DEFI schemes due to absolute lymphopenia of the patient, one patient with BACH2 mutation and one patient with NFKB1 mutation both identified as CID CVID due to low numbers or percentage of naïve CD4 T cells.

In summary, the different definitions for CID among adult CVID patients strongly overlap but are not interchangeable. While specificity for DRCs, lymphoma and mortality didn't vary significantly between the alternative definitions, the sensitivity was remarkably higher when considering the relative naïve CD4 T-cell reduction < 10% as previously discussed by Giovannetti et al. [8] and proposed in this manuscript. Absolute total or naïve CD4 T-cell reduction correlated best with panlymphopenia and may therefore indicate a distinct pathomechanism affecting lymphocyte homeostasis in general. γδ-T cell expansion, absent T-cell proliferation (data not shown), severe shift in the CD4-TCR Vβ repertoire, or bona-fide CID-related mutations identified only single patients. These patients suffer very likely from CID and not CVID and should be identified by the respective tests. Interestingly, except for those with a skewed CD4 repertoire, these patients were not sufficiently captured by any of the current CID definitions. Thus, despite an extensive genetic search, which is an essential part of the diagnostic work up of these patients, a reliable detection of patients with CID within CVID continues to be challenging; especially since most of the suggested criteria for CID are based on quantitative T-cell parameters and we are still missing informative assays assessing T-cell (dys) function beyond their proliferative capacity.

For all adult CVID patients without a clear diagnostic marker of CID, the relative reduction of naïve CD4 T cells below 10% was the most sensitive indicator to suggest some cellular immunodeficiency. This alteration of CD4 homeostasis is associated with a higher prevalence of DRCs, lymphoma and death, but cannot be separated from other CVID patients based on their genetic profile or additional immune phenotypic alterations. Therefore, we suggest that these patients currently remain within the CVID cohort but are carefully observed as a subgroup of 'CVID patients with a T-cell defect' for transition to CID and the need of more extensive clinical care.

3. Methods

Clinical and laboratory data recorded between 2001 and 2017 from 271 adult patients with CVID diagnosis [1], who were registered in the patient data base of the University Clinic Freiburg, were analyzed retrospectively. All patients provided written informed consent and the study was approved by the Ethics Committee University Medical Center Freiburg (No: 282/11).

Data from patients with current systemic immunosuppressive therapy (except prednisolone or equivalent < 10 mg/d) and from patients after chemotherapy or after stem cell transplantation were excluded; 238 patients were included into the analysis. Median follow-up was 7 years (interquartile range 3–10 years) after diagnosis. Consecutive measurements over two or more years were available of 201 (CD4 T-cell counts) and 142 patients (naïve CD4 T-cell percentages). The most recent CD4 T cell or naïve (CD45RA+) CD4 T cell counts, as well as the history of opportunistic infections (according to table 4.23 in [11]) were used to allocate the CVID patients to the respective CID definitions.

DEFI2009-LOCID: CD4 T cells < 200/μl for at least two years or history of opportunistic infection.

DEFI2015-LOCID: naïve (CD45RA+) CD4 T cells < 20/μl or history of opportunistic infection.

Freiburg-CID: naïve (CD45RA+) CD4 T cells < 10% or history of opportunistic infection.

ESID2014-CID: CD4 T cells < 200/μl and naïve (CD45RA+) CD4 T cells < 10%.

Data for T-cell proliferation after PHA and anti-CD3 or anti-CD3/CD28 stimulation (being part of the ESID2014-CID criteria) was available for 47 patients. Absent T-cell proliferation was detected but not confirmed in three patients, while in all other patients, proliferation was normal. Because of the missing confirmation, these data were not included and the classification according to ESID2014-CID was modified as mentioned above. All patients not fulfilling any of the four CID definitions were referred to as 'non-CID CVID patients'. Clinical parameters were summarized in Table 1, immunological parameters in Table S1. Disease related complications (DRCs) were defined according to Bertinchamp et al. [4] and included enteropathy, autoimmune cytopenia and granulomatous disease; lymphoma was analyzed separately.

One-way ANOVA with Dunn's multiple comparisons post-hoc test using GraphPad Prism for Windows, GraphPad Software, La Jolla California USA, www.graphpad.com, was used for comparisons between the non-CID CVID patients and the different CID groups ($*p < .05$, $**p < .01$, $***p < .001$, $****p < .0001$).

Whole exome sequencing (WES) data was available for 86 patients and screened for mutations in CID-, antibody deficiency-, or immune dysregulation-related genes according to tables 1, 3 and 4 in [10].

The raw sequenced reads stored in FASTQ files were mapped against the human reference genome build UCSC hg19 using Bowtie 2 v2.2.3 [12], reordered, sorted and converted to BAM format, followed by the removal of PCR duplicates with Picard v1.115 (http://picard.sourceforge.net). Local realignment around InDels and base quality score recalibration as well as variant calling and variant quality score recalibration were performed with the GATK v3.1 [13] according to their best practice recommendations. Genetic variation data stored in VCF format files was handled using VCFtools program package [14]. For the annotation of discovered variants with the IDs from the short

genetic variations database dbSNP v142 (http://www.ncbi.nlm.nih.gov/SNP/) the program SnpSift was used, which is part of the main distribution of the toolbox SnpEff v3.6 (http://snpeff.sourceforge.net) by [15]. The annotation of variants with the genes and transcripts they are affecting and the effects they produce was conducted using the effect prediction tool SnpEff.

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Declarations of interest

None of the authors has any conflict of interest related to the content of this manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clim.2019.04.001.

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