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ORIGINAL ARTICLE

Gammadelta T cells as a predictor of surgical relapse of Crohn's disease

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KEYWORDS
Crohn's Disease;
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Summary
Background: We recently demonstrated a decrease in the overall lymphocyte population in the peripheral blood of patients with CD compared to healthy controls and this decrease is more evident in γδ T lymphocytes. The percentages of T cell subsets could reflect the risk of surgical relapse in CD patients. The aim of this study is to study the correlation between αβ and γδ T cell subsets in the peripheral blood of patients with CD and the risk for surgery during follow up.
Methods: A prospective study of 102 patients with CD compared with 102 healthy subjects (control group) matched by age and sex was conducted. Lymphocytic populations of CD3+, CD4+, CD8+, CD56+, and αβ and γδ T cell subsets were measured in the peripheral blood of all participants.
Results: We found evidence of a relationship between lower γδ T cell levels and risk of surgical relapse in CD. The lowest subsets observed in CD patients with surgical relapse were CD3 + γδ, CD3 + CD8 + γδ and CD3 + CD56 + γδ T cells. We observed a relationship between a decrease in γδ T cells and the most severe forms of the disease. The lowest levels of CD3 + γδ and CD3 + CD8 + γδ T cells were observed in the fistulizing phenotype.

Abbreviations: CD, Crohn's disease; TCR, T cell receptor; CDAI, Crohn's Disease Activity Index.

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Conclusions: The deficit of γδ T cells was related with the severity and the risk for surgical relapse in CD patients. Patients with CD3 + γδ deficit were more prone to surgery than patients without this deficit. These results suggest that γδ T cells could be used as markers of poor prognosis of CD following the diagnosis of the disease.

Introduction

Crohn's disease (CD) is a chronic relapsing inflammatory disease primarily involving the small and large intestine, but which can also affect other parts of the gut. CD is characterized by recurring flares and the occurrence of complications like fistulas and abscesses is common during the disease course, often requiring surgery. There is widespread agreement on the use of the term ''relapse'' to refer to a flare of symptoms while ''recurrence'' is used to define the reappearance of lesions after surgical resection [1].

Some clinical predictors are well established in CD: age below 40 years, presence of perianal disease at diagnosis, need for steroids to treat the first flare, presence of upper gastrointestinal lesions, terminal ileal location and ileo-colonic lesions are all predictive of a first surgical operation and disabling disease [2—6]. Serological and genetic predictors of CD have also been studied [7], but only a few studies looked at the role of lymphocytes as predictors of poor prognosis in CD. In the 80s, two studies investigated the role of peripheral lymphocytes in the prediction of recurrence in CD and concluded that lymphopenia is indeed a predictor of recurrence [8,9].

Lymphocytes can be distinguished according to their TCR as αβ or γδ T cells [10]. γδ T cells scarce in the peripheral blood but are abundant in the intestinal mucosa where they act as first line of defense against pathogens due to their plasticity. In addition, γδ T cells play an important role in tissue repair and protection [11—14]. γδ T lymphocytes, probably the predominant intraepithelial lymphocytes subset in the intestinal mucosa, mediate both regulatory and pathogenic roles and are capable of producing both pro-inflammatory cytokines (IFN-γ and TNF-α) and anti-inflammatory cytokines (TGF-β and IL-10) [14,15]. γδ T cell depletion or deficiency leads to increased inflammation in experimental murine models of inflammatory bowel disease, because γδ T cells act as regulatory T cells *in vivo* by the induction of anti-inflammatory cytokines involved in epithelial regeneration. Aggravation of intestinal inflammation and mortality by deficiency of γδ T cells in different types of inflammatory bowel disease animal models has been observed [16—18].

However, γδ T cells can promote the generation of gut antigen reactive effector cells and generate colitogenic effector T cells that contribute to developing severe intestinal inflammation [14]. CD103 + $\alpha4\beta7^{high}$ γδ T cells have been proposed as novel subset of ''inflammatory'' γδ (iγδ) T cells, that may promote chronic inflammation in the intestine [19].

We recently demonstrated that there is a decrease in the overall lymphocyte population in the peripheral blood of patients with CD compared to healthy controls and this decrease is more evident in γδ T lymphocytes. Furthermore, γδ T lymphocytes were lower in all clinical scenarios of CD studied: remission, active disease and new diagnosed patients [20].

The aim of this study is to investigate if the determination of the different T cell subsets (αβ and γδ) in peripheral blood could predict the risk of suffering surgical complications in CD patients after follow-up.

Materials and methods

Study population

One hundred and two patients with CD were included in this prospective study from June 2007 to July 2015. We recruited prospectively CD patients admitted to the emergency and gastroenterology departments (both newly diagnosed and patients with active disease) and CD patients in clinical remission controlled at the outpatient gastroenterology clinic. Lennard-Jones criteria were used for the diagnosis of patients with CD [21]. Disease activity was measured using the Crohn's Disease Activity Index (CDAI) [22]. Three different clinical scenarios were defined: ''new patients'' were patients with active CD presenting at, or shortly after, diagnosis with no previous treatment for CD; patients in ''remission'' were patients with a CDAI $\leq$ 150 for at least 12 months; and patients with ''active disease'' were patients with a CDAI $\geq$ 150 and signs and symptoms of disease.

Blood donors were recruited (control group), matched with the patient group by sex and age (± 5 years). Relatives of CD patients were excluded. Additional selection criteria for control subjects were: absence of acute infections, inflammatory, autoimmune, or immune-deficiency diseases, and no immunosuppressive or antibiotic treatment or any kind of vaccine during the previous year. Surgical relapse was defined by the need for any surgical intervention in the course of the disease, including surgical interventions for perianal complications and any other major abdominal surgery related with the disease (resections, strictureplasty etc.).

Each participant in the study signed an informed consent form, and the study was approved by the Ethics and Investigation Committee of the Arnau de Vilanova Hospital, Valencia, Spain.

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Methods of blood sample analysis

Whole blood was stained using direct immunofluorescence and simultaneous quadruple labeling with the following monoclonal antibodies: CD4, CD8, CD56, CD3, TCR$\alpha\beta$ and TCR$\gamma\delta$, for the T $\alpha\beta$ and $\gamma\delta$ lymphocyte study. Monoclonal antibodies were conjugated with fluorescein isothiocyanate (FITC), phycoerythrin (PE), phycoerythrin-Texas red (ECD), and R-phycoerythrin-cyanine 5 (PC5). The $\gamma\delta$ T lymphocyte populations were analyzed with PC5 conjugated anti-human $\gamma\delta$ TCR (Beckman Coulter, Miami, USA [clone: IMMU510]). The $\alpha\beta$ T lymphocyte populations were analyzed with PC5 conjugated anti-human $\alpha\beta$ TCR (Beckman Coulter [clone: IP26A]). Fluorescence analysis was performed using a Beckman-Coulter multiparameter flow cytometry analyzer, Cytomics FC 500, Florida (USA) and later analyzed with CXP Software. A minimum of 30,000 events were measured.

Statistical analysis

When normality was assumed (Kolmogorov–Smirnov test), Student's *t* or ANOVA tests were used to compare the mean values of the quantitative variables. When the hypothesis of normality for the quantitative variable was not accepted, non-parametric tests were used.

The frequencies of cell subsets did not show a normal distribution. When values were transformed into Napierian logarithms, they reached the normal distribution. The normal values of our population were calculated, in healthy subjects of a previous study, [23] using the equation: Exponent (mean Napierian logarithm $\pm$ 1.96 * standard deviation Napierian logarithm) (Table 2).

The Kaplan–Meier survival method was used to estimate the cumulative probability of clinical relapse or need for surgery according to the deficit of T cell subsets. Differences between curves were tested using the Log-Rank test (Statistical program R, version 3.3). A Cox proportional hazards regression model was used to assess whether clinical variables were independently associated with the risk of need for surgery. P value < 0.05 was considered statistically significant.

Results

Patient population

Two hundred and four subjects were included in the study, 102 patients with CD (45 female and 57 male) and 102 healthy controls (45 female and 57 male). The mean age of patients with CD was 39.1 ± 13.9 years (range 15 to 80 years) and the mean age of controls was 39.5 ± 14.7 years (range 8 to 78 years), $P = 0.815$. The mean age of male patients with CD was 40.5 ± 15 vs. 37.8 ± 13.3 in females, $P = 0.187$. In the healthy control group, the mean age of males was 40.4 ± 14.9 years vs. 38.4 ± 13.1 in females, $P = 0.501$. There was no significant difference in age between genders. Patient characteristics are presented in Table 1.

Table 1 Clinical characteristics of the study cohort (Crohn's disease patients).

	Mean	SD
Harvey-Bradshaw Index	6.9	3.7
Crohn's Disease Activity Index	199.8	110.1
	N	%
Surgical relapse (SR)	16	15.7
Intraoperative diagnosis of SR	16	100.0
Fistula (2 perianal fistula)	8	50.0
Stenosis	4	25.0
Inflammatory mass	4	25.0
Clinical scenarios		
New Patient	33	32.4%
Remission	33	32.4%
Active disease	36	35.3%
Montreal age		
A1 (< 16)	8	7.8%
A2 (17-40)	67	65.7%
A3 (> 40)	27	26.5%
Montreal Location		
L1 (ileal)	43	42.2%
L2 (colonic)	15	14.7%
L3 (ileocolic)	44	43.1%
Montreal Behavior		
B1 (inflammatory)	61	59.8%
B2 (stenotic)	21	20.6%
B3 (fistulizing)	20	19.6%

T cell subsets and deficiency in Crohn's Disease and control

The mean values of total lymphocytes in CD patients were $1.7 \pm 0.7 \times 109$ vs. $2.2 \pm 0.6 \times 109$ in the group of healthy controls, $P < 0.001$. With respect to the Montreal Classification, significant differences were found between the different phenotypes (Monte Carlo test): inflammatory vs. fistulizing ($1.7 \pm 0.6 \times 10^9$ and $1.4 \pm 0.8 \times 10^9$, respectively, $P = 0.035$) and stenotic vs. fistulizing ($1.8 \pm 0.6 \times 10^9$ and $1.4 \pm 0.8 \times 10^9$, respectively, $P = 0.036$).

The T cell subsets in patients with CD compared to the control group are shown in the Fig. 1. There was a decrease in the overall T cells subsets in the peripheral blood of patients with CD compared to healthy controls ($P < 0.001$). This decrease was more evident in $\gamma\delta$ T cells, and this reduction was largely attributable to a decrease in CD3 + CD8 + $\gamma\delta$ and CD3 + CD56 + $\gamma\delta$ T cells. Significant differences were not seen in the case of $\alpha\beta$ T cells. According to the Montreal classification, a significant decrease was observed in the disease behavior (B) only in the $\gamma\delta$ T cells (Fig. 2). CD3+ $\gamma\delta$ and CD3 + CD8+ $\gamma\delta$ T cells were significantly lower ($p = 0.036$ and $p = 0.014$, respectively) (Bonferroni test) in the fistulizing disease compared to the other phenotypes (stenotic and inflammatory).

The differences in T cell subsets in patients with CD compared to the control group are shown in Table 2. Reference values and cell deficit criteria are shown in the Material and Methods section and in Table 2. We found a statistically significant reduction CD3+, CD3 + CD4+ and CD3 + CD8+ $\gamma\delta$ T cells in CD patients compared to healthy subjects. In

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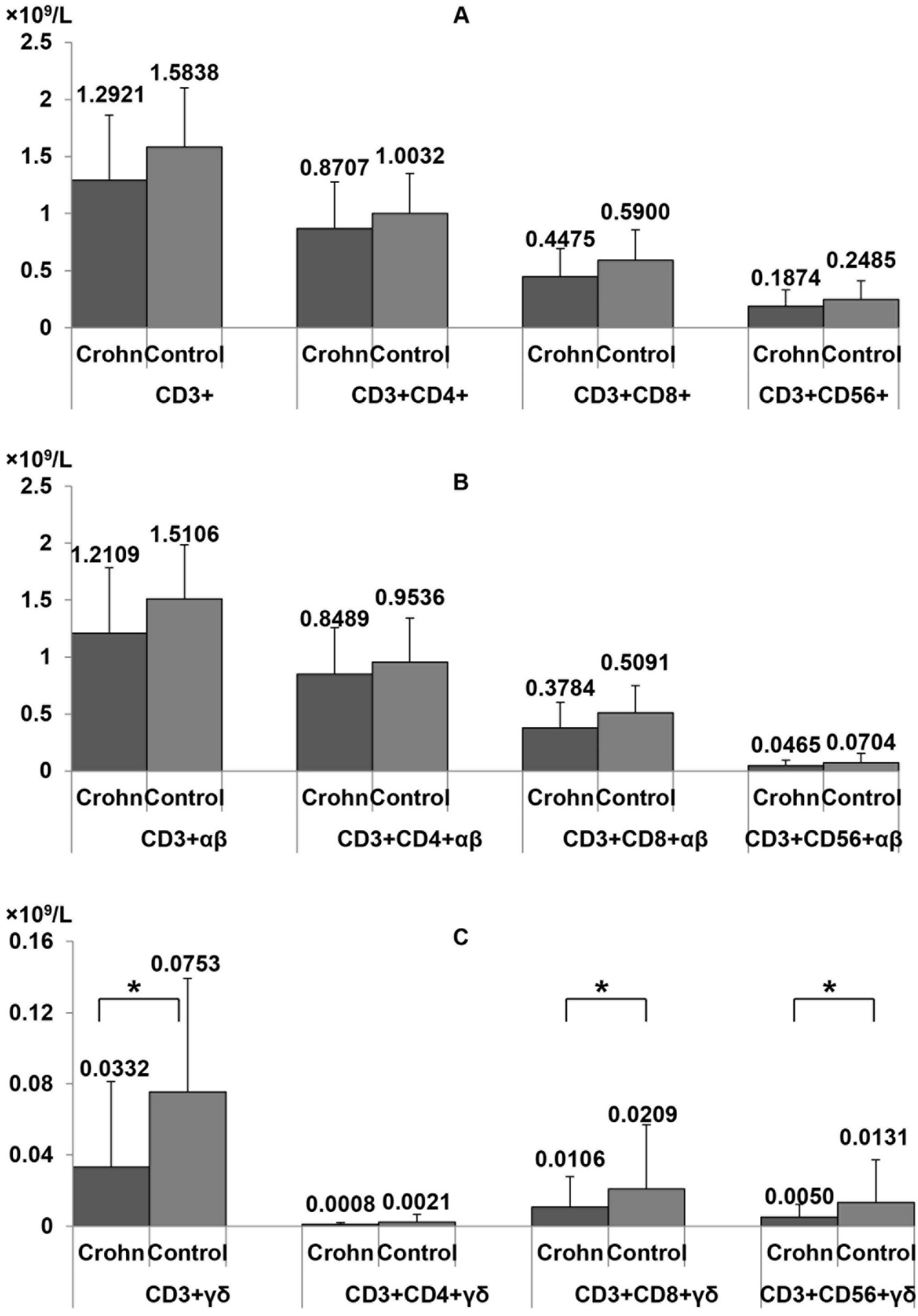

Figure 1 Comparative frequency (mean) of T cell subsets in Crohn's patients and control subjects. Panel A: T cell subsets; panel B: αβ T cell subsets; panel C: γδ T cell subsets. Values are expressed as means ($\times 10^9$/Liter), and T bars denote standard deviation. Mann–Whitney *U* test, (*$P < 0.001$).

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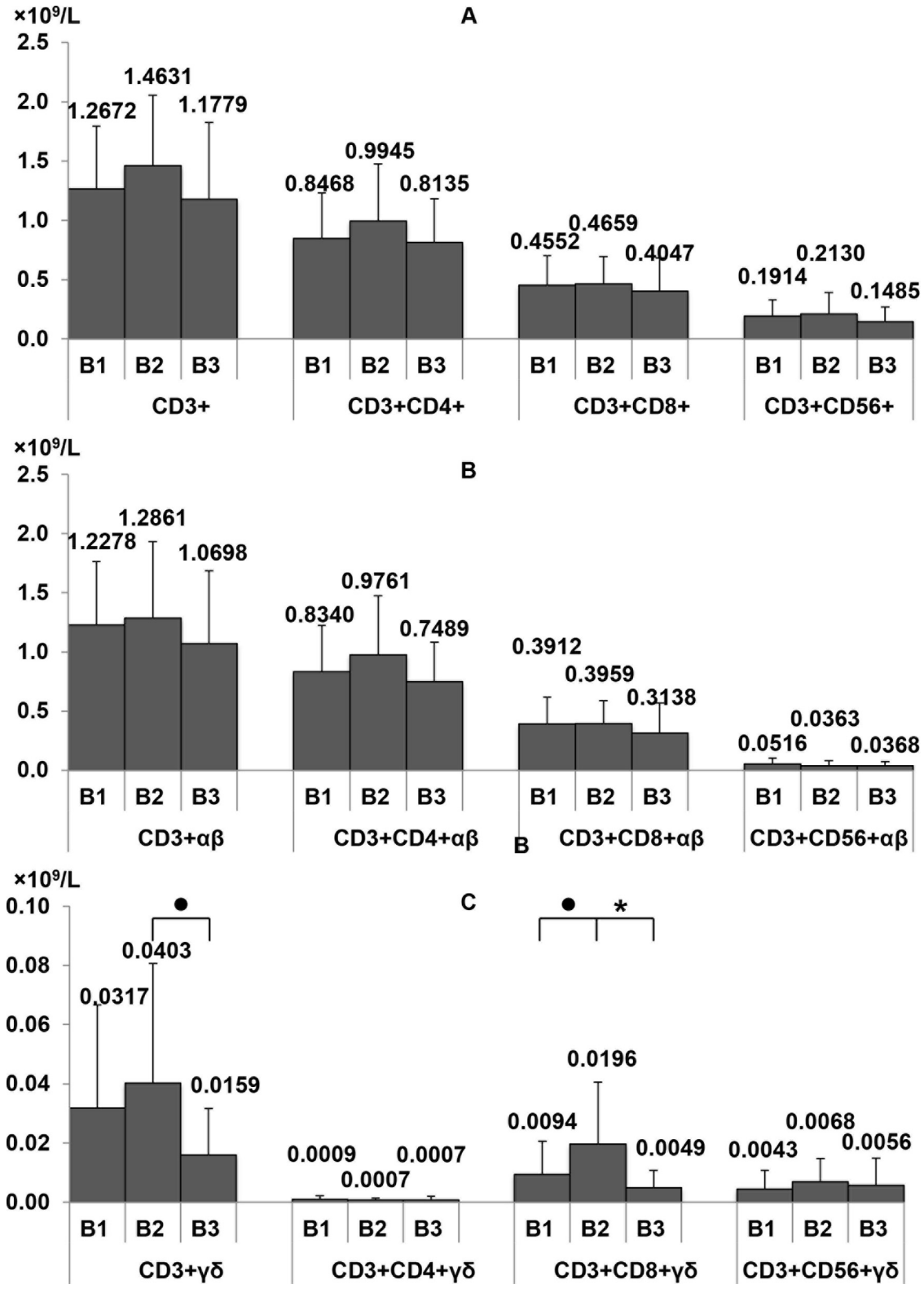

Figure 2 Comparative frequency (mean) of T cell subsets in Crohn's patients according to Montreal Classification. Panel A: T cell subsets; panel B: αβ T cell subsets; panel C: γδ T cell subsets. B1: inflammatory; B2: stenotic; B3: fistulizing. ANOVA (Bonferroni test) *$P < 0.001$; $P < 0.05$. Values are expressed as means (× 109/Liter), and T-bars denote standard deviation.

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Table 2 Deficit of T cell subsets in patients with Crohn's disease.

	Crohn $n = 102$		Control $n = 102$			
Deficit (Reference-range/mm^3)	n	%	n	%	OR (95% CI)	Sig.(p)
CD3+ (720.5—3,070.1)	14	13.7%	2	2.0%	7.0 (1.6—30.1)	0.003
CD3 + CD4+ (408.2—2,105.2)	7	6.9%	3	2.9%	2.3 (0.6—8.8)	NS
CD3 + CD8+ (236.2—1,443.2)	21	20.6%	4	3.9%	5.3 (1.9—14.8)	< 0.001
CD3 + CD56+ (49.3—1,008.5)	12	11.8%	1	1.0%	12.0 (1.6—90.6)	0.003
CD3 + αβ (651.7—2,853.5)	6	5.9%	6	5.9%	1.0 (0.3—3.0)	NS
CD3 + CD4 + αβ (375.1—2,065.8)	4	3.9%	4	3.9%	1.0 (0.3—3.7)	NS
CD3 + CD8 + αβ (137.2—1,365.7)	8	7.8%	2	2.0%	4.0 (0.9—18.4)	NS
CD3 + CD56 + αβ (4.3—453.0)	6	5.9%	2	2.0%	3.0 (0.6—14.5)	NS
CD3 + γδ (7.0—297.8)	24	23.5%	5	4.9%	4.8 (1.9—12.1)	< 0.001
CD3 + CD8 + γδ (1.3—90.6)	12	11.8%	3	2.9%	4.0 (1.2—13.8)	0.029
CD3 + CD56 + γδ (0.3—85.1)	8	7.8%	3	2.9%	2.7 (0.7—9.8)	NS

Reference range of healthy adult subjects ($n = 157$) from a previous study (23). OR (95% CI): Odds ratio (95% Confidence Interval). NS: Not Significant.

contrast, no differences were observed in the αβ T cells subpopulations between patients and controls. Within the γδ population, CD3 + γδ and CD3 + CD8 + γδ T cells showed the greatest difference in CD patients vs. the control group ($P < 0.001$ and $P = 0.029$, respectively).

Clinical stages and T cells subsets

Fig. 3 shows the frequency of T cell subsets in CD patients according to clinical scenarios: new patient ($n = 33$), remission ($n = 33$) and active disease ($n = 36$).

A significant decrease in CD3 + CD8+ T cell subsets was observed in CD patients, both in total T cells and in αβ and γδ subsets. CD3+ γδ T cells were significantly decreased with active disease compared to patients in remission. The risk of suffering a surgical intervention during follow up was higher in patients with active disease 8/36 (22.2%) and new patient 6/33 (18.2%) than in patients in remission 2/33 (6.1%).

T cell subsets and deficiency in surgical relapse

Of the 102 CD patients studied, 16 needed a surgical intervention during follow up. T cell subsets frequencies were analyzed in order to establish a relation with the risk of surgery (Fig. 4). The percentages of CD3 + γδ, CD3 + CD8 + γδ and CD3 + CD56 + γδ subsets were significantly lower in CD patients that needed surgery, ($P < 0.001$, $P = 0.001$ and $P = 0.048$, respectively).

The correlation between T cells subsets and the need for surgery was analyzed with Cox regression bivariate analysis (Hazard Ratio adjusted). The deficit of CD3 + γδ and CD3 + CD8 + γδ T subsets was statistically significant in patients who needed surgery vs. those who did not (Table 3).

The Kaplan-Meier curve for the risk of surgical relapse among patients with deficit of CD3 + γδ T cells and CD3 + CD8 + γδ T cells is shown in Fig. 5. Patients with CD3 + γδ and CD3 + CD8 + γδ T cell deficit had higher percentages of surgical relapse (Fig. 5, panels A and B, respectively). The risk showed an increase at 60 months. The mean time to surgical relapse was shorter among CD3 + γδ T cells deficient patients than patients with no deficit in CD3 + γδ T cells 56.8 months (CI95%, 45.6—68.3) vs. 73.7 (CI95%, 67.0—80.7), $P = 0.020$. Also, CD3 + CD8 + γδ T cells deficient patients had a shorter time to the first surgical intervention, 52.9 months (CI95%, 38.7-67.2) vs. 72.3 (CI95%, 65.8-78.9), $P = 0.022$. Furthermore, patients with a deficit of CD3 + CD8 + γδ T cells had a higher risk of surgical relapse on a multivariate analysis (Table 4).

Medical treatment and surgical relapse

Adjusted Hazard Ratio (HR) (95% CI) for treatment-associated risk factors for surgical relapse were analysed by univariate and multivariate Cox regression analysis (Tables 4 and 5). With the multivariate Cox regression analysis using the forward conditional selection, both CD3 + CD8+ γδ T cell deficit and corticosteroid treatment were the only risk factors associated with the occurrence of surgical relapse. Both CD3 + CD8+ γδ T cell deficit and corticosteroid treatment were independent variables with no significant differences between their HR (< 10%). No significant interaction effect was observed between both variables ($P = 0.597$).

Discussion

This study demonstrates for the first time a relationship between lower γδ T cell levels and the risk of surgical relapse in CD. Most patients experience a worsening of CD during in the natural history of CD, with some studies showing that less than 12% of CD patients remain free of a clinical relapse in the first 10 years after diagnosis [24]. In the present work, 15.68% of our patients suffered surgical relapse in the period of study (from June 2007 to July 2015).

This study confirms the results obtained in a previous work [20], in which we demonstrated that patients with CD have a reduced number of lymphocytes compared to healthy subjects, now in a larger sample of patients (102 vs. 40).

The decline of all T cell subsets, both αβ and γδ T cells, was widespread. However, the decrease of γδ T cells was

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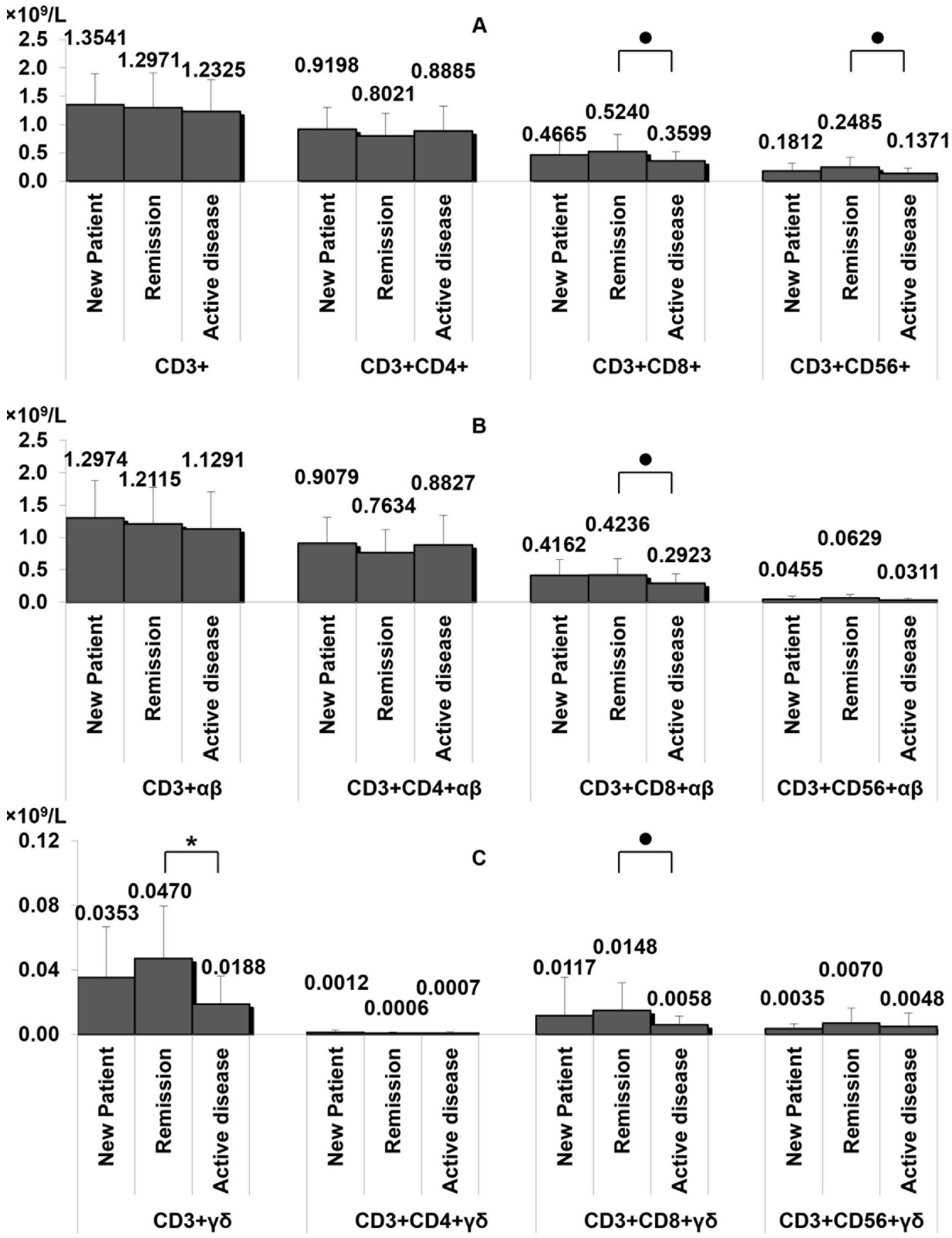

Figure 3 Comparative frequency (mean) of T cell subsets in Crohn's patients according to disease stages: New patient ($n = 33$), Remission ($n = 33$) and Active disease ($n = 36$). Panel A: conventional subsets; panel B: αβ T cell subsets; panel C: γδ T cell subsets. Values are expressed as means (× 109/Liter), and T-bars denote standard deviation. ANOVA (Bonferroni test). *$P < 0.001$; $P < 0.05$.

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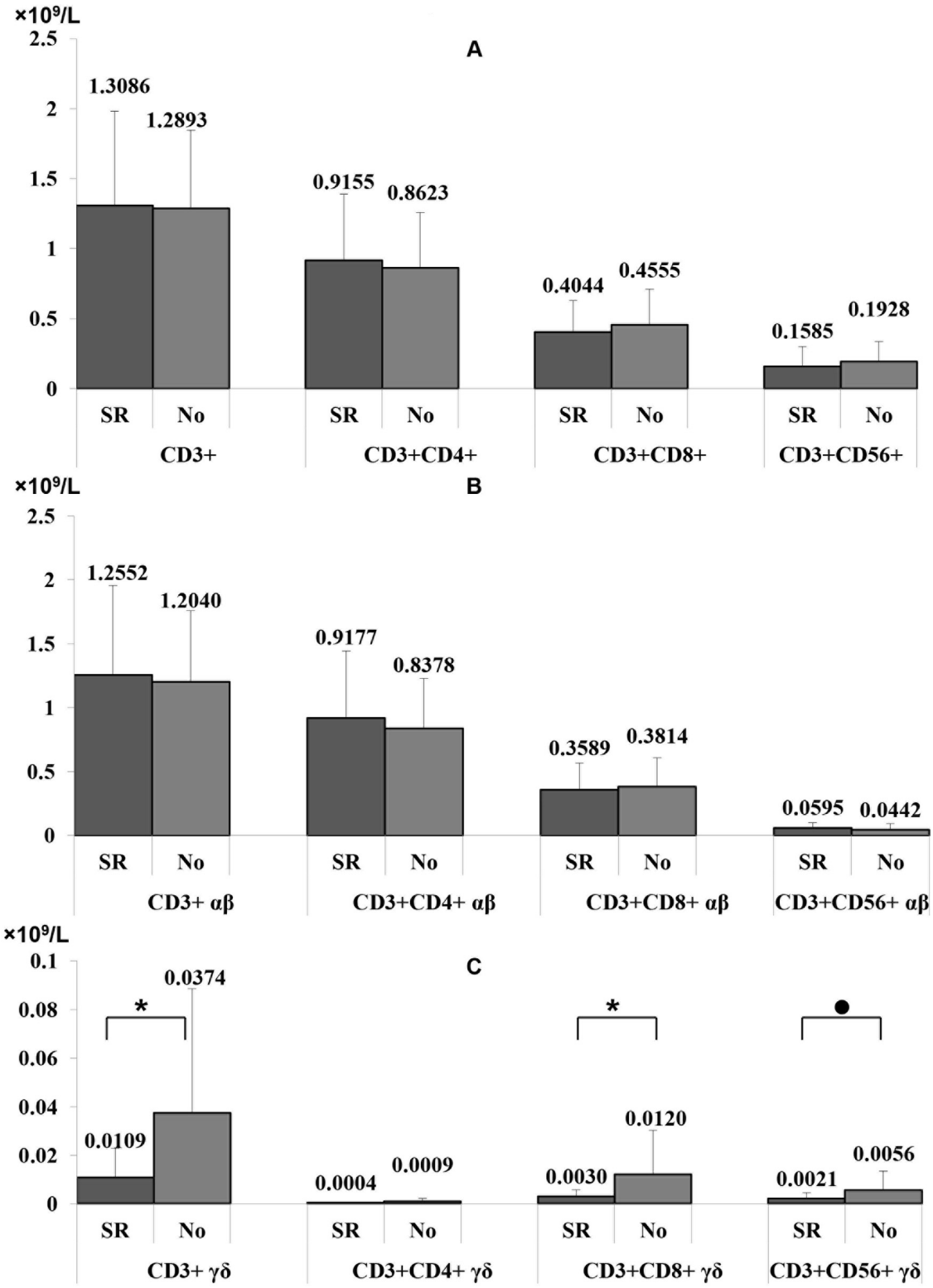

Figure 4 Comparative Frequency of T cell subsets (mean) in CD patients according to surgical relapse (SR, $n = 16$) vs. patients without surgical relapse (No, $n = 86$). Panel A: conventional T cell subsets; panel B: αβ T cell subsets; panel C: γδ T cell subsets. Mann—Whitney U test. *$P < 0.001$. $P < 0.05$. Values are expressed as means ($\times 10^9$/Liter), and T-bars denote standard deviation.

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Table 3 Deficit of T cell subsets related with surgical relapse. Bivariate Cox Regression model analysis.

		B	S.E.	Wald	df	Sig.	Exp (B) HR adjusted	I.C. 95% Exp(B)	
								Lower	Higher
Step 1	Deficit CD3+ T cells	0.613	0.643	0.909	1	0.340	1.847	0.523	6.515
Step 1	Deficit CD3 + CD4+ γδ T cells	1.258	0.771	2.659	1	0.103	3.519	0.776	15.961
Step 1	Deficit CD3 + CD8+ γδ T cells	0.817	0.549	2.220	1	0.136	2.264	0.773	6.635
Step 1	Deficit CD3+ αβ T cells	0.570	0.648	0.773	1	0.379	1.768	0.496	6.297
Step 1	Deficit CD3 + CD4+ αβ T cells	0.778	1.040	0.559	1	0.454	2.176	0.284	16.695
Step 1	Deficit CD3 + CD8 αβ T cells	-0.044	1.039	0.002	1	0.966	0.957	0.125	7.329
Step 1	Deficit CD3 + CD56+ αβ T cells	0.471	1.038	0.205	1	0.650	1.601	0.209	12.251
Step 1	Deficit CD3+ γδ T cells	1.135	0.513	4.905	1	0.027	3.112	1.140	8.496
Step 1	Deficit CD3 + CD8+ γδ T cells	1.116	0.520	4.576	1	0.032	3.053	1.098	8.488
Step 1	Deficit CD3 + CD56+ γδ T cells	0.740	0.646	1.315	1	0.251	2.097	0.592	7.431

B: Beta coefficient, S.E.: Standard Error, Wald statistic, df: degrees of freedom, Exp (B): Exponential Beta, sig: significance, HR (Hazard Ratio), CI: Confidence Interval.

Table 4 Treatment related with Surgical Relapse. Multivariate Cox Regression model analysis.

		B		S.E.		Wald		df		Sig.		Exp (B) HR adjusted		CI 95% Exp (B)	
		Inferior		Superior		Inferior		Superior		Inferior		Superior		Lower	Higher
Step 1	Corticosteroid	1.336		0.511		6.823		1		0.009		3.803		1.396	10.361
Step 2	Deficit CD3 + CD8+ γδ T cell	1.123		0.519		4.684		1		0.030		3.073		1.112	8.495
	Corticosteroid	1.358		0.518		6.860		1		0.009		3.886		1.407	10.734
Step 1	Corticosteroid * Deficit CD3 + CD8+ γδ T cell	-0.578		1.092		0.280		1		0.597		0.561		0.066	4.773

B: Beta coefficient, S.E.: Standard Error, Wald statistic, df: degrees of freedom, Exp (B): Exponential Beta, sig: significance, HR (Hazard Ratio), CI: Confidence Interval.

Table 5 Treatment related with surgical relapse. Bivariate Cox Regression model analysis.

		B		S.E.		Wald		df		Sig.		Exp (B) HR adjusted		IC 95% Exp (B)	
		Inferior		Superior		Inferior		Superior		Inferior		Superior		Lower	Higher
Step 1	Azathioprine	0.766		0.503		2.324		1		0.127		2.152		0.803	5.763
Step 1	Anti-TNF	0.133		0.758		0.031		1		0.860		1.143		0.259	5.049
Step 1	Mesalazine	−0.068		0.505		0.018		1		0.893		0.934		0.347	2.514
Step 1	Salazopyrine	−3.043		7.601		0.160		1		0.689		0.048		0.000	140686.268
Step 1	Antibiotic	1.328		0.502		6.994		1		0.008		3.775		1.410	10.104
Step 1	Corticosteroid	1.336		0.511		6.823		1		0.009		3.803		1.396	10.361
Step 1	Methotrexate	1.667		1.099		2.298		1		0.130		5.294		0.614	45.661

B: Beta coefficient, S.E.: Standard Error, Wald statistic, df: degrees of freedom, Exp (B): Exponential Beta, sig: significance, HR (Hazard Ratio), CI: Confidence Interval. TNF: Tumor Necrosis Factor.

more marked, stressing the importance of γδ T cells in the pathogenesis of CD, as predicted by previous experimental IBD studies [16–18]. In the present study, the lowest subsets observed in CD patients with surgical relapse were CD3 + γδ, CD3 + CD8 + γδ and CD3 + CD56 + γδ.

Several decades ago, Heimann TM et al. [8] showed that CD patients with marked preoperative lymphocytopenia were more likely to develop early symptomatic recurrence after surgical intervention than patients with normal lymphocyte counts.

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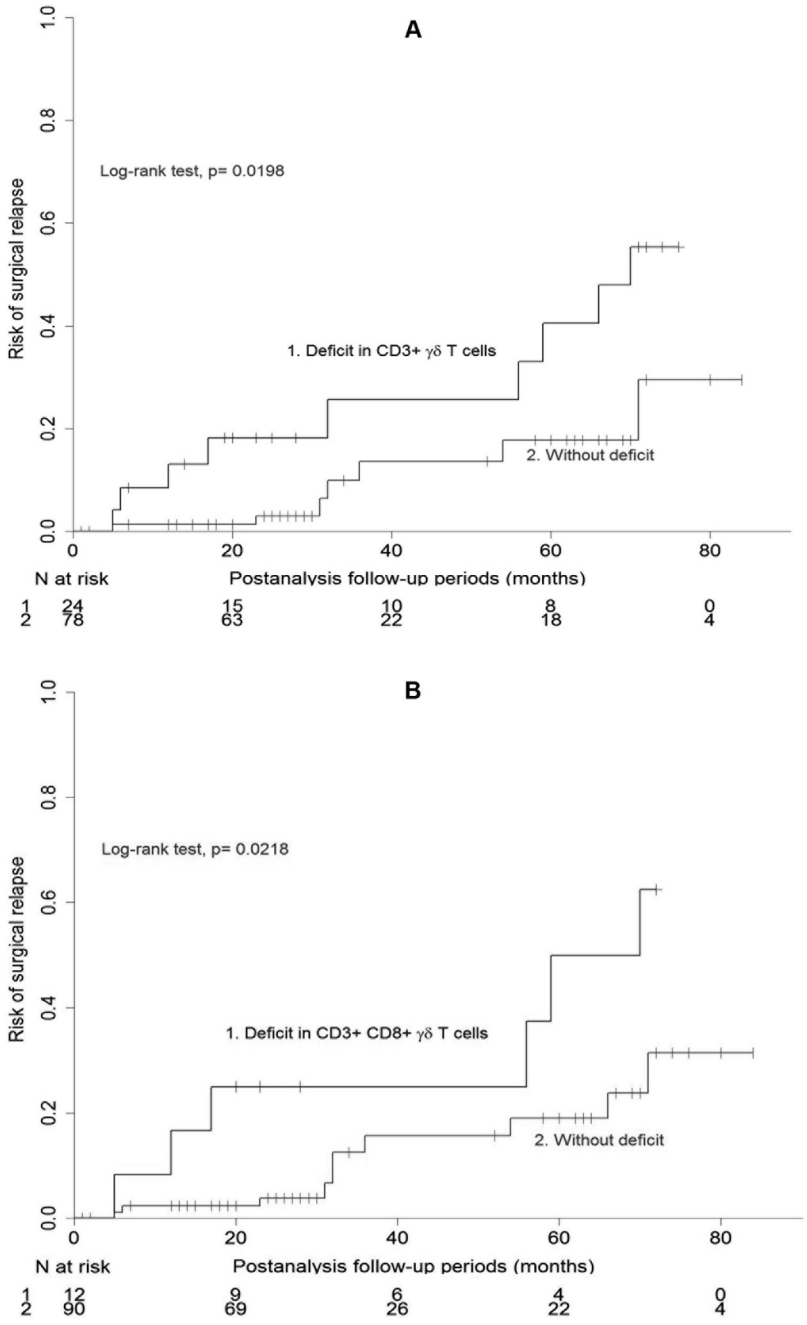

Figure 5 Kaplan—Meier plots illustrating risk of surgical relapse following CD3 + γδ T cells deficiency (A) and CD3 + CD8 + γδ T cells deficiency (B) in peripheral blood of Crohn's disease patients. Time is expressed in months (mean).

In our work lymphocyte counts were significantly lower in patients with CD compared to healthy subjects. Although there were no significant differences between the different phenotypic patterns of CD, there was a tendency toward a decrease in total lymphocytes in the fistulizing phenotype (B3), one of the most aggressive types in the natural history of CD. This lack of significance may be due to the small number of cases in this group. In the above-mentioned studied by Heimann TM et al. correlations were observed between the disease process in the intestine and the peripheral lymphocyte count, suggesting that preoperative lymphopenia could reflect the patient's inability to regulate the inflammatory process [8].

Nevertheless, the most interesting result of our work is that although all the T cell subsets were decreased in patients with CD, only the γδ T cell populations were significantly decreased in patients with surgical relapse. Conversely, no differences were seen in the αβ T cells.

γδ T cells are involved in epithelial cell production and wound repair by stimulating the production and deposition of hyaluronic acid by epithelial cells, and increasing reparation and tissue healing by the production of key

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keratinocyte growth factors, like KGF 1 and KGF 2, and IGF-1 [25,26]. The γδ T cell deficit in CD could therefore cause changes in the healing process of ulcers as well as fistulas in these patients. However, other mechanisms explaining this decrease in peripheral blood should be also investigated.

Our results confirm the relation between a decrease in γδ T cells and the most severe forms of the disease. As commented before, this is of special interest in the fistulizing phenotype, where the lowest CD3+ γδ and CD3 + CD8+ γδ T cell levels were observed.

Selective loss of circulating Vδ2 T cells has been previously reported in azathioprine-treated CD patients [27]. In our study, the percentage of patients treated with azathioprine was slightly higher in the group requiring surgery than in those who did not suffer surgical complications (22.5% versus 12.9%, NS), but this difference was not statistically significant. In addition, there were no significant differences in the total number of patients treated with azathioprine in both groups (surgical relapse or non-surgical relapse). Opposite to that, the deficit in γδ T cells was clear and significant in the surgical relapse group. In fact, CD3 + CD8+ γδ T cell deficit and corticosteroid treatment were the only risk factors associated with surgical relapse in a multivariate analysis.

Besides, we did not observe any significant differences of γδ T cells when comparing treated and untreated patients. Therefore, we think that although azathioprine could influence the reduction in γδ T cells, it is not the main factor causing the deficit observed in these patients.

These data could indicate that the deficit of γδ T cells is what contributes to surgical relapse, since all patients with surgical relapse have low γδ T cell values.

In summary, γδ T cells are significantly decreased in CD, especially in the fistulizing phenotype of the disease, reflecting greater severity in this subgroup of patients. The deficit of γδ T cells was also related with the probability of suffering a surgical relapse of CD. Our findings showed that patients with CD3 + γδ deficit were more prone to surgery than patients without deficit in the follow-up. These results suggest that γδ T cells could be used as markers of a poor prognosis of CD as of the moment of diagnosis of the disease if they were routinely determined at the time of admission.

Authors contributions

The conception and design of the study: Andreu-Ballester JC, Catalan-Serra I. Acquisition of data: Catalan-Serra I, Gil-Borrás R, Marqués-García P. Citometry analysis: García-Ballesteros C and López-Chuliá F. Analysis and interpretation of data: Andreu-Ballester JC, Catalan-Serra I, Cuellar C. Drafting the article or revising it critically for important intellectual content: Andreu-Ballester JC, Catalan-Serra I, Cuellar C. Final approval of the version to be sumited: Andreu-Ballester JC, Catalan-Serra I, Cuellar C.

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Ethics, consent, and permissions

All study participants provided written informed consent to the use of their clinical data and blood samples. The study protocol was approved by the Ethic Committee of Arnau de Vilanova Hospital, Valencia (Spain).

Disclosure of interest

The authors declare that they have no competing interest.

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