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ORIGINAL ARTICLE

Multidimensional inflammatory and immunological endotypes of idiopathic focal segmental glomerulosclerosis and their association with treatment outcomes

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ABSTRACT

Objectives. Idiopathic focal segmental glomerulosclerosis (FSGS) has been linked to immunological and inflammatory response dysregulations. The aim of this study was to find endotypes of FSGS patients using a cluster (CL) analysis based on inflammatory and immunological variables, and to analyse whether a certain endotype is associated with response to treatment with corticosteroids.

Methods. This prospective observational study included patients with idiopathic FSGS diagnosed by kidney biopsy. Serum levels of soluble interleukin (IL)-1 receptor, tumoural necrosis factor alpha, Interferon gamma (IFNγ), IL-6, IL-17, IL-12, IL-23, IL-13, IL-4, IL-5, IL-6, haemopexin (Hx), haptoglobin (Hgl), soluble urokinase-type plasminogen activator receptor (suPAR) and urinary CD80 (uCD80) were measured with enzyme-linked immunosorbent assay or nephelometry. T-helper lymphocyte populations and T-regulatory lymphocytes were analysed by flow cytometry. A factorial analysis followed by a *k*-means CL analysis was performed.

Results. A total of 79 FSGS patients were included. Three CLs were identified. CL1 (27.8%) included IL-12, IL-17, IL-23 and a T helper 17 (Th17) pattern. CL2 (20.2%) included IL-4, IL-5, IL-13, immunoglobulin E and Th2 pattern. CL3 (51.8%) included IL-6, Hx, Hgl, suPAR and uCD80. There were no differences in age, gender, kidney function, albumin or proteinuria among CLs. About 42/79 patients (53.1%) showed cortico-resistance. The prevalence of cortico-resistance was significantly lower in CL2 (4/16, 25%) than in CL1 (16/26, 72.7%) and CL3 (22/41, 53.7%) ($P = 0.018$), with no significant differences between CLs 1 and 3 ($P = 0.14$).

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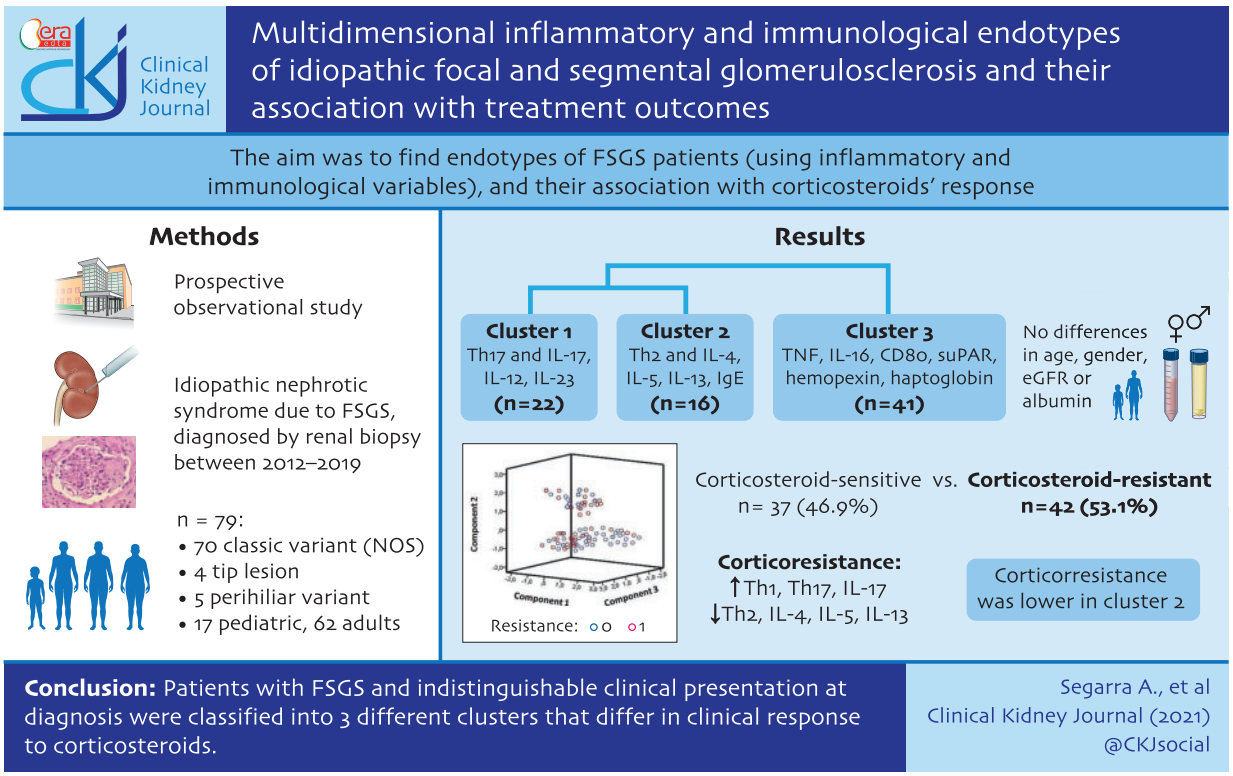
Conclusions. Patients with FSGS and indistinguishable clinical presentation at diagnosis were classified in three distinct CLs according to predominant Th17, Th2 and acute inflammatory responses that display differences in clinical response to treatment with corticosteroids.

Keywords: cluster analysis, endotypes, focal segmental glomerulosclerosis, idiopathic nephrotic syndrome, inflammatory response, interleukins, lymphocyte populations

GRAPHICAL ABSTRACT



INTRODUCTION

The term idiopathic focal segmental glomerulosclerosis (FSGS) refers to a clinicopathological entity that has diverse aetiologies and pathogenic mechanisms, and a common histopathological expression characterized by the presence of sclerosis/hyalinosis lesions in a variable percentage of glomeruli and affecting only one part of them [1]. This morphological definition allows a heterogeneous group of patients to be included under this same term. The response to corticosteroids has been identified as the main long-term outcome variable [2], but it has been found that many patients do not respond well to the treatment with corticosteroids or immunosuppressant drugs based on current classification, supporting the notion that FSGS is a multifactorial disorder with diverse and overlapping pathologies, sharing an identical phenotype at the time of diagnosis. In contrast to the phenotypic classifications, endotyping categorizes disease variants based on their underlying pathophysiologic mechanisms [3]. Therefore, identification of FSGS endotypes, determined by distinct pathophysiological mechanisms, and further characterizing the FSGS uncontrolled by current treatment regimens may provide a basis to understand disease causality and to develop efficient management approaches. The pathogenesis of FSGS is still unknown and has been linked to the presence of a circulating soluble factor capable of inducing irreversible podocyte injury [4, 5]. Since some patients respond to treatment with corticosteroids and/or immunosuppressants [1, 2], it has been suggested that, in certain cases, the pathogenesis of FSGS may be related to the activation of the inflammatory and/or the immune response [6]. Studies analysing the inflammatory and immunological response in patients with nephrotic are scarce, mainly focused on minimal change disease (MCD) and have led to discordant results [7–11]. In some of them, an increase in the levels of interleukin (IL)-6, IL-1 and tumour necrosis factor α (TNFα) [4, 9, 11] has been described, but in others, the levels of these molecules were not higher than those of healthy controls [7, 8, 10]. Some recent studies found a relationship between FSGS and certain cytokines of inflammatory origin, like soluble urokinase-type plasminogen activator receptor (suPAR) [12, 13] or cardiotrophine-like cytokine-1 [14], and others described a potential role for TNFα [15] and transforming growth factor-β (TGF-β) [16]. The role of lymphocytes in the pathogenesis of idiopathic nephrotic syndrome (INS) has been the focus of multiple investigations mainly focused on MCD [4, 5, 17], which have found different patterns of T helper (Th) response. Some studies

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have not shown differences between Th1/Th2 ratio [18], but others have found a predominance of a Th2 pattern [19, 20] and had even provided evidence that IL-13, related to a Th2 response, is capable of inducing podocyte injury in experimental models [19]. In all cases, these relationships have been inconclusive. Instead of these 'pathogenic-driven' approaches, no studies have used unstructured approaches that incorporate multi-dimensional data to identify clusters (CLs) of patients with presumably similar mechanisms of disease.

The aim of this study was to find endotypes of patients with FSGS by integrating multidimensional inflammatory and immunological variables, using a CL analysis, and to analyse the association between these endotypes and clinical response to treatment with corticosteroids.

MATERIALS AND METHODS

Study design and population

This prospective observational study, included patients with INS due to FSGS, diagnosed by renal biopsy between 2012 and 2019 in five tertiary referral hospitals. We included both paediatric and adult patients, who met all the following inclusion criteria: (i) diagnosis of FSGS performed by renal biopsy, containing more than eight glomeruli; (ii) absence of secondary aetiologies after conducting a systematic and protocolized study; (iii) absence of a family history of nephropathy; and (iv) no treatment with steroids, immunosuppressants, angiotensin II blockers or statins at the time blood and urine samples were obtained. Patients with collapsing glomerulopathy were excluded.

INS was defined by 24-h urinary protein excretion values >3.5 g/day in adults and >40 mg/m^2/h in children associated with hypalbuminaemia <3.5 g/dL and oedema. After diagnosis, both the clinical follow-up and the treatment criteria were carried out following the recommendations indicated in the KDIGO 2012 guidelines. The criteria for complete and partial remission were also defined according to those described in these guidelines, both for paediatric and adult patients [13].

Idiopathic FSGS was diagnosed with the evidence of typical lesions in optical microscopy associated with diffuse podocyte effacement in electron microscopy and after exclusion of secondary aetiologies including: reduction of renal mass, morbid obesity, HIV-associated nephropathy, heroin or cocaine use, parvovirus B19 infection, consumption of analgesics, bisphosphonates or interferon, vesicoureteral reflux or obstructive sleep apnoea. A genetic study to rule out pathogenic genetic mutations was performed in all patients who showed corticoresistance and were <35 years old, using a kidney panel previously described and validated [21]. Patients carrying known pathogenic mutations were excluded from the study.

All serum samples were obtained during the nephrotic phase before starting any treatment. Blood samples were obtained in standard tubes with no additives, were centrifuged at 1500g × 10 min and stored at −80°C until used. Serum creatinine was measured by the IDMS-traceable compensated method (Hitachi Modular P-800 Roche Diagnostics, Germany). The estimated glomerular filtration rate (eGFR) was calculated using the Schwartz equation in children and Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula in adults. Hemopexin (Hx) and haptoglobin (Hgl) were measured by nephelometry (Coulter Biotek, Berlin, Germany). Urinary CD80 (uCD80) was measured by ELISA [Human CD80 ELISA Kit (B7-1) (ab256392), Cambridge, UK]. The serum levels of IL-6 and suPAR were measured by ELISA (Quantikine R&D Systems, Inc., MN, USA). The level of TNFα and interferon-γ (IFNγ), IL-17, IL-12 and IL-23, were measured using the MILLIPLEX® MAP system (catalogue number HCYTOMAG-60K Millipore Corp., MO, USA). All ELISA analyses were performed in duplicate. In addition, to analyse the reproducibility of the measures, in a sample of 15 patients, three or more determinations were made during the nephrotic outbreak phase, prior to the start any treatment. In all cases, the variation coefficients of repeated measures were <10%.

The Th subtypes and T-regulatory (Treg) cells were analysed from peripheral blood samples using total heparinized blood without performing any manipulation other than the incubation with the different monoclonal antibodies. Total blood samples were incubated at 37°C with the monoclonal antibodies conjugated with different fluorochromes, at the corresponding dilution and concentration and were analysed by flow cytometry using the following monoclonal antibodies: anti-CD3, anti-CD4, anti-CXCR3, anti-CCR4, anti-CCR6, anti-CD25, anti-hCD127, anti-FoxP3 and anti-CD45RO (FACSCanto, Becton Dickinson, San Jose, CA, USA). Th subtypes and Treg cells were defined according to the staining patterns summarized in Supplementary Methods, Table S1.

Histopathological analysis of kidney biopsies

All kidney biopsies were blindly analysed by two experienced nephropathologists who confirmed the morphological pattern of FSGS case by case. Kidney biopsies were stained with haematoxylin and eosin, periodic acid–Schiff-methenamine and Masson's trichrome for morphological analysis and immunofluorescence studies were carried out with antibodies against immunoglobulin A (IgA), IgG, IgM, C3, fibrinogen and light chains, and were processed for an electron microscope study.

Statistical analysis

Quantitative variables are expressed as mean ± standard deviation. For dichotomous variables, a Chi-squared test was performed to determine the difference between groups. Data distribution was tested for normality using a Kolmogorov–Smirnov test or a Shapiro–Wilk test. Analysis of variance was used to assess significant intergroup variability among more than two groups and unpaired two-tailed Student's t-test was used for between-group comparisons. Significance was accepted at $P < 0.05$, which was adjusted using a Bonferroni correction for multiple comparisons. We analysed 23 different variables related to inflammatory response, serum IL levels and Th cell polarization. Principal component analysis was performed to analyse relationships among variables to choose the variables most relevant to the study. We retained significant components with eigenvalue ≥1. For CL analysis, continuous variables were standardized using z scores. Orthogonal rotation with Kaiser normalization was performed, and only variables with loadings of >0.4 were retained. Next, the subjects were sorted into groups by using a k-means method based on the correlation ratio and mixed principal component analysis (PCA). The optimal number of CLs was determined by NbClust in R software package. Imputation of the missing values was performed with the k-Nearest Neighbour method and iterative robust regression using the VIM package. Patients and variables with >10% of missing data were removed. After classifying the patients according to the response to corticosteroid treatment, a univariate analysis was performed to analyse the differences

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between the two groups. The cut-off values of quantitative variables associated with the response to treatment were calculated by receiver operating characteristic (ROC) curves, using Youden's index to select the optimal value. The relationship between the response to corticosteroids and the type of CL was analysed by Chi-squared test.

This study adhered to the parameters established by the declaration of Helsinki. All patients gave their informed consent in writing and the bioethics committee of the corresponding centre approved the study.

RESULTS

Patient selection

Ninety-eight patients were screened; however, 19 patients (19.4%) were excluded because of the following reasons: nine patients (9.2%) received steroids, immunosuppressants, angiotensin II receptor antagonists or statins, three patients (3%) did not give their written consent, three (3%) patients were lost to follow-up within the first 3 months after diagnosis and four patients (4%) carried known pathogenic mutations in genes encoding proteins of glomerular filtration barrier (NPHS2: $n = 1$; collagen IVα3: $n = 2$ and LMB1X: $n = 1$). The final study group included 79 patients. Of these patients, 17 (21.5%) were paediatric and 62 (71.5 %) were >18 years. Seventy patients showed the classic variant (NOS), four patients showed a tip lesion and five patients showed a perihiliar variant (Figure 1).

Clinical and biochemical characteristics

The main clinical and biochemical characteristics of the study group are summarized in Table 1. The correlation matrix among biochemical variables is shown in Supplementary Results, Table S2.

Principal component analysis

We first performed a PCA to reduce dimensionality of variables from a total of 23. Five components were retained, explaining 73.1% of all variance in the data. Table 2 summarizes the results of the PCA and the percentage of variability retained by each of those five components. Table 3 summarizes the variables included in each component in the rotated component matrix. Variables with scores <0.4 are not represented. Component 1 comprised the percentage of Th2 lymphocytes and the levels of IL-4, IL-13 and IgE. Component 2 included the percentage of Th17 lymphocytes, the circulating levels of IL-17, IL-23 and IL-12 and the percentage of regulatory T lymphocytes. Component 3 included levels of TNFα, IL-6, Hx, soluble IL-1 receptor (sIL1R) and Hgl. Component 4 included IFNγ and Component 5 included suPAR. Figure 2 shows the 3D scatter-plot graphic representation of the distribution of all these variables for the first three PCA that retained 59.1% of total variability.

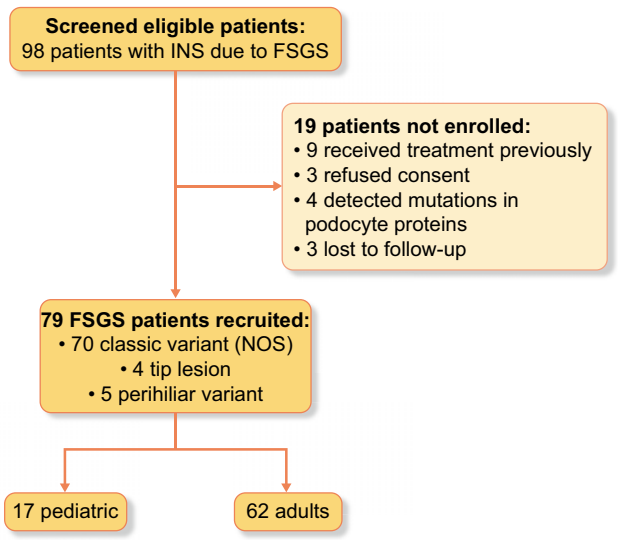

FIGURE 1: Flow chart of patient selection.

Table 1. Clinical and biochemical characteristics of FSGS patients

Variables	FSGS patients $n = 79$
Age, median (IQR), years	35 (25–49)
Gender: male, *n* (%)	37 (46.8)
Steroid resistance, *n* (%)	42 (53.2)
Creatinine, mg/dL	1.1 (0.86–1.42)
eGFR, mL/min/1.73 m^2	88 (74.4–104.4)
Proteinuria, g/dL	10.8 (8.2–14.4)
Albumin, g/dL	2.48 (2–2.78)
Th1, %	23 (22–25)
TNFα, pg/mL	8.8 (5.4–14.8)
IFNγ, pg/mL	14.4 (7.7–24.4)
IL-12, pg/mL	664.5 (478–774)
Th2, %	34 (31–39)
IL-4, pg/mL	3.6 (2.1–6.1)
IL-5, pg/mL	4.5 (2.2–6)
IL-13, pg/mL	4.1 (2.4–6.4)
IgE, g/dL	49 (46–98)
Th17, %	3.3 (2.4–24.5)
Th1–Th17, %	6.5 (3.9–9.8)
Treg, %	2.1 (1.4–3)
IL-17, pg/mL	7.2 (3–31)
IL-23, pg/mL	35.6 (25.6–51.6)
sIL1R, pg/mL	2257 (1383.9–3085.4)
IL-6, pg/mL	3.9 (1.8–12.7)
Hx, mg/mL	110.9 (49.1–324.9)
Hgl, mg/dL	137.8 (90.8–338.8)
suPAR, ng/mL	3421.8 (2409.6–4830)
uCD80, ng/mg creat	36 (16.4–56)

Values are expressed as median [interquartile range (IQR)] 25–75th quartile unless otherwise specified.

CL analysis

Figure 3 represents the graphic classification dendogram of patients in three differentiated CLs. Table 4 shows the distribution of the biochemical variables analysed, in each and one of these three CLs. The results of the analyses of variance to compare the distribution of biochemical variables among CLs are summarized in Supplementary Results, Table S3. There were no differences in age, gender, kidney function, albumin or proteinuria among the three CLs. There were no significant differences between adult and paediatric patients (Supplementary Results, Table S1). CL1 included 22 patients (27.8%) and was characterized by a significantly higher percentage of both Th17 lymphocytes and circulating levels of IL-17, IL-12 and IL-23, as well as a percentage of regulatory T lymphocytes significantly lower than those of CL2 and CL3. CL2 included 16 patients (20.3%) and was characterized by a percentage of Th2 lymphocytes and levels of IL-4, IL-5, IL-13 and IgE significantly higher than those of CL1

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Table 2. Results of PCA and percentage of variability retained by each component for the five components with eigenvalues >1

	Initial eigenvalues			Extraction sums of squared loadings			Rotation sums of squared loadings		
Component	Total	% of variance	Cumulative %	Total	% of variance	Cumulative %	Total	% of variance	Cumulative %
1	6.152	30.762	30.762	6.152	30.762	30.762	4.746	23.729	23.7
2	3.792	18.962	49.724	3.792	18.962	49.724	3.616	18.080	41.8
3	2.185	10.923	60.647	2.185	10.923	60.647	3.473	17.364	59.1
4	1.357	6.785	67.432	1.357	6.785	67.432	1.438	7.190	66.3
5	1.139	5.694	73.126	1.139	5.694	73.126	1.353	6.763	73.1

Table 3. Rotated component matrix showing the variables retained in each component.

	Component				
Variables	1	2	3	4	5
Th2	0.923	–	–	–	–
IL-4	0.908	–	–	–	–
IL-13	0.852	–	–	–	–
IgE	0.749	–	–	–	–
Th17	−0.639	0.785	–	–	–
IL-23	–	0.774	–	–	–
IL-12	–	0.753	–	–	–
Tregs	–	−0.670	–	–	–
IL-17	–	0.651	–	–	–
Th1	–	−0.532	–	–	–
IL-6	–	–	0.818	–	–
Hx	–	–	0.800	–	–
sIL1R	–	–	0.765	–	–
TNFα	–	–	0.748	–	–
Hgl	–	–	0.720	–	–
IFNγ	–	–	–	0.809	
suPAR	–	–	–	–	0.867

Variables with scores <0.4 are not represented.

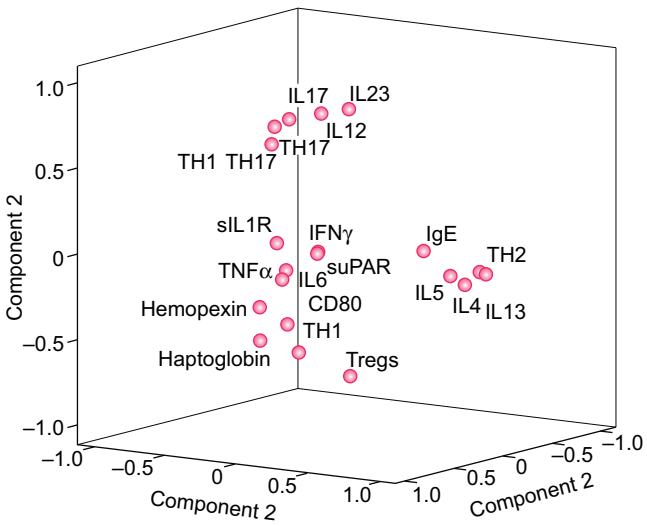

FIGURE 2: 3D scatter-plot graphic representation of the distribution of variables according to the first three PCA that retained the 59.1% of total variability.

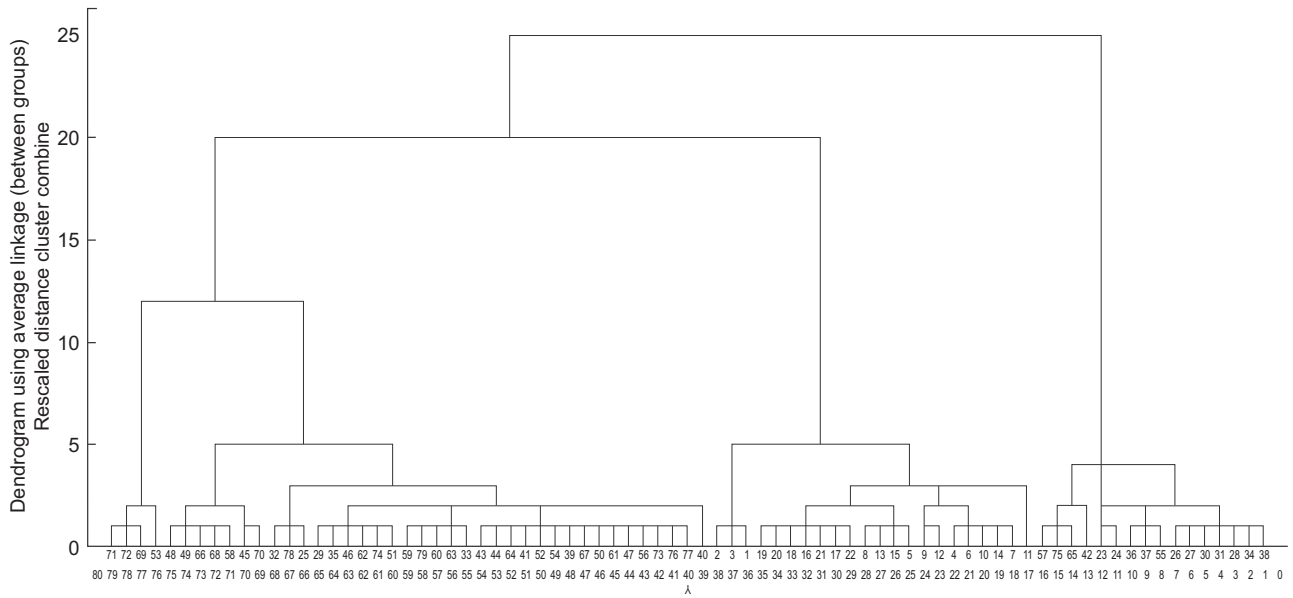

FIGURE 3: Classification dendogram of patients showing the existence of three differentiated CLs.

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Table 4. Characteristics of FSGS patients according to CL differentiation

Variables	CL1, $n = 22$	CL2, $n = 16$	CL3, $n = 41$	P1	P2	P3
Clinical characteristics						
Age, years	34.5 (28–47.5)	39 (32.5–58)	34.5 (16–46.5)	0.755	1	0.404
Cortico-resistance, *n* (%)	16 (72.7)	4 (25)	22 (53.7)	0.018	0.140	0.018
Biochemical characteristics						
Creatinine, mg/dL	1.1 (0.75–1.3)	1 (0.8–1.4)	1.1 (0.9–1.4)	1	1	1
eGFR, mL/min/1.73 m^2	84.9 (74.1–97.3)	90 (81.8–113.8)	87.9 (73.6–104.4)	1	0.818	1
Proteinuria, g/dL	10.1 (7.8–13.2)	13.3 (9.1–18)	9.3 (8.2–14)	0.207	1	0.155
Albumin, g/dL	2.44 (2.18–2.6)	2.15 (1.5–3.3)	2.49 (2.2–2.8)	1	1	1
Th1, %	23 (20–24)	22 (22–26)	23.5 (22–25.7)	1	**0.015**	0.063
TNFα, pg/mL	5.85 (4.9–7.5)	5.4 (2.9–7.3)	14.1 (10.8–18.7)	1	**0.000**	**0.000**
IFNγ, pg/mL	12 (4.9–26)	10.5 (4–16.9)	16.7 (10.3–24.4)	1	1	1
IL-12, pg/mL	760 (709–881.8)	580 (504.5–665)	552 (348–771)	**0.000**	**0.000**	0.403
Th2, %	32.5 (30–37.3)	61 (37–67.5)	34 (32–37)	**0.000**	1	**0.000**
IL-4, pg/mL	4.3 (2.7–4.9)	13 (4.6–14.8)	2.65 (2–3.9)	**0.000**	1	**0.000**
IL-5, pg/mL	3.5 (2–4.7)	6.9 (2.3–8.6)	4.6 (2.4–5.7)	**0.000**	1	**0.000**
IL-13, pg/mL	4.2 (3.7–5.6)	11 (4.4–15.3)	3.7 (1.3–5.2)	**0.000**	0.710	**0.000**
IgE, mg/mL	47 (45–48)	143 (48.5–160)	50.5 (45.2–98)	**0.000**	0.292	**0.000**
Th17, %	30.9 (25.1–34.7)	3 (2.1–3.8)	3 (2.1–3.7)	**0.000**	**0.000**	1
Th1–Th17, %	13.3 (7.2–17.6)	5.6 (2–8.6)	5 (3.8–8.7)	**0.000**	**0.000**	0.506
Treg, %	0.8 (0.6–1)	2.9 (2.1–3.2)	2.5 (1.9–3.7)	**0.000**	**0.000**	1
IL-17, pg/mL	47.1 (38.1–54.9)	4.7 (2.3–8)	4.7 (2.8–7.9)	**0.000**	**0.000**	0.737
IL23, pg/mL	57.3 (35.1–63.6)	37 (25.3–48.8)	32 (23–42)	**0.000**	**0.000**	1
sIL1R, pg/mL	2294.9 (1496.2–2916.6)	1857.8 (1162.1–2345)	2296.2 (1328.5–3483)	1	1	1
IL-6, pg/mL	3 (1.8–3.9)	2 (1.2–5.6)	12.4 (3.9–19.6)	1	**0.002**	**0.011**
Hx, mg/mL	48.4 (28.4–72.7)	49 (40.1–116.5)	320.6 (136.9–440.4)	0.374	**0.000**	**0.001**
Hgl, mg/dL	69 (33.6–127.3)	116.8 (61.8–213.1)	321.4 (147.05–425.3)	0.543	**0.000**	**0.000**
suPAR, ng/mL	3413.5 (2497.8–4765.9)	3421 (2703.3–4830)	3444 (2373–4672.5)	1	1	0.647
uCD80, ng/mg creat	32.3 (16.1–45.2)	32.7 (6–56.5)	37.2 (23–78.5)	1	**0.029**	**0.008**

Values are expressed as median (interquartile range) 25–75th quartile unless otherwise specified. The bold values are the p values with stadistical significance ($p<0.05$).

Table 5. Patient characteristics according to the response to corticosteroid treatment

Variables	Cortico-sensitive, $n = 37$	Cortico-resistant, $n = 42$	P-value
Age, years	34 (26–38)	41 (23.3–54)	0.34
Gender: male, *n* (%)	21 (56.8)	16 (43.2)	0.09
Creatinine, mg/dL	1 (0.9–1.2)	1.2 (0.8–1.5)	0.12
eGFR, mL/min/1.73 m^2	90 (81.8–107.6)	85.9 (73.4–99.4)	0.13
Proteinuria, g/dL	12.1 (8.2–14.5)	9.9 (8.4–12.8)	0.34
Albumin, g/dL	2.56 (2–2.8)	2.41 (2–2.6)	0,85
Th1, %	24 (22–26.5)	22.5 (21–24.3)	0.13
TNFα, pg/mL	8.7 (4.5–14.7)	9 (5.7–15.1)	0.62
IFNγ, pg/mL	14.4 (9.5–26.2)	12.8 (5–24)	0.28
IL-12, pg/mL	618 (483–716.5)	726 (466–834)	**0.046**
Th2, %	36 (30.5–59)	34 (31.8–37.3)	**0.043**
IL-4, pg/mL	3.6 (2.4–11.6)	3.6 (1.9–4.6)	**0.007**
IL-13, pg/mL	4.8 (2.4–9.5)	3.9 (2–5.8)	0.12
IL-5, pg/mL	4.2 (2.2–7.3)	4.5 (2–5.9)	0.13
IgE, mg/mL	51 (46.5–143)	47 (45–77.3)	0.16
Th17, %	3.1 (2.1–6.3)	4.3 (2.4–25.9)	0.18
Th1–Th17, %	6.5 (3.5–9.2)	6.5 (4.3–14.7)	0.07
Treg, %	2.7 (1.8–3.1)	1.8 (0.8–2.6)	0.06
IL-17, pg/mL	4.8 (2.3–9)	9.8 (4.4–38.5)	**0.023**
IL-23, pg/mL	33 (23–46)	43 (29.8–56.9)	**0.043**
sIL1R, pg/mL	2133.1 (1141.1–2916.6)	2257.1 (1615.1–3185.7)	0.63
IL-6, pg/mL	2.7 (1.2–8.5)	10.7 (3–17)	0.83
Hx, mg/mL	110.9 (46.3–152.9)	114.5 (49–364.7)	0.18
Hgl, mg/dL	116.8 (70.8–321.4)	139.3 (99.5–371)	0.37
suPAR, ng/mL	3421.8 (2407.5–4915.9)	3206.7 (2457.1–4124.6)	0.34
uCD80, ng/mg creat	34 (15.5–55.5)	36.5 (17.6–81.8)	0.17

Values are expressed as median (interquartile range) 25–75th quartile unless otherwise specified. The bold values are the p values with stadistical significance ($p<0.05$).

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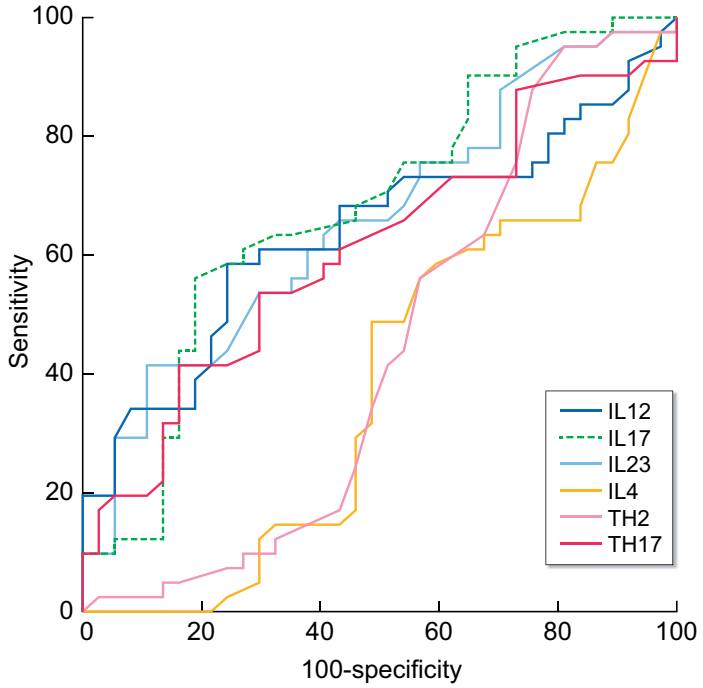

Variable	AUC	SE	95% CI	Cut-off value	Sensitivity (95% CI)	Specificity (95% CI)
IL12 pg/mL	0.638	0.0641	0.522 to 0.744	> 698	58.5 (42.1–73.7)	75.6 (58.8–88.2)
IL23 pg/mL	0.658	0.0619	0.542 to 0.762	> 49	40.5 (25.6–56.7)	89.1 (74.6–97)
IL4 pg/mL	0.619	0.0654	0.502 to 0.726	≤ 4.9	83.3 (68.6–93)	45.9 (29.5–63.1)
IL17 pg/mL	0.681	0.0617	0.566 to 0.782	> 9.2	57.1 (41.0–72.3)	81 (64.8–92)
TH2 (%)	0.562	0.0690	0.445 to 0.674	≤ 38	83.3 (68.6–93)	43.2 (27.1–60.5)
TH17 (%)	0.612	0.0644	0.495 to 0.720	> 9.35	40.5 (25.6–56.7)	83.8 (68.0–93.8)

FIGURE 4: ROC curves to analyse the capacity of variables that showed significant differences in the univariate analysis to predict cortico-resistance.

and CL3. CL3 included 41 patients (51.9%) and was characterized by the absence of polarization of Th lymphocyte subpopulations and by levels of TNFα, IL-6, Hx, Hgl, suPAR and CD80 significantly higher than those of CL1 and CL2.

Variables associated with the response to treatment with corticosteroids

Overall, 42/79 patients (53.1%) showed corticosteroid resistance. Table 5 summarizes the baseline characteristics of patients, classified according to the response to corticosteroid treatment. There were no differences in age, gender, eGFR or albumin between cortico-sensitive and cortico-resistant patients. Patients with cortico-resistance showed higher levels of IL-17, Th17 and Th1–Th17 cells and lower levels of Th2 cells and IL-4, IL-5 and IL-13 than patients with cortico-sensitivity. Figure 4 shows the results of ROC curves performed to analyse the capacity of each of these variables to predict cortico-resistance, including the area under the curve and their associated Youden's values. The prevalence of cortico-resistance was significantly lower in CL2 (4/16, 25%) than in CL1 (16/22, 72.7%) and CL3 (22/41, 53.7%) ($P = 0.018$). Figure S1 in the Supplementary Results shows a 3D graphic representation of the distribution of patients according to their respective factor scores for the first three PCA, differentiating cortico-sensitive and cortico-resistant cases. In this graphic representation, it can also be seen that patients with high scores for Component 1, corresponding to a Th2 pattern, showed a significantly lower frequency of cortico-resistance.

DISCUSSION

The results of our study indicate that, at the time of diagnosis and in the absence of pharmacological interferences, the inflammatory response and the polarization of Th cell subtypes do not follow a single pattern common to all patients with idiopathic FSGS. From the 23 variables initially analysed, our patients could be classified into three CLs with well-differentiated signatures. CL1 can be considered as a predominant Th17 cell response, with lower percentage of Treg cells and higher levels of IL-17 and IL-23. CL2 is characterized by a predominant Th2 response pattern, with increased levels of IL-4, IL-5, IL-13 and IgE, and CL3 comprised patients with no polarization of T-cell response and with increased levels of proteins and mediators of the acute inflammatory response. When comparing these three CLs, there were significant differences in both inflammatory and immunological profiles among them, but no differences in the clinical profile of the disease at the time of diagnosis in terms of age, gender distribution, serum albumin, eGFR or proteinuria.

The role of lymphocytes in the pathogenesis of INS has been analysed in several studies [4, 5, 17, 22, 23]. Most of them have focused on MCD and have found different types of polarization

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of the Th response. Some studies have not shown differences in Th1/Th2 ratio [18], whereas others have found a predominance of a Th2 pattern [19, 20] and had even provided evidence that IL-13, related to a Th2 response, is capable of inducing podocyte injury in experimental models [19]. In our cohort, ∼20% of patients (CL2) showed such a predominant Th2 response. This type of response was not the most prevalent, but showed a frequency of cortico-resistance significantly lower than that observed in CL1 and CL3. These results are in agreement with previously published data in which an association between a predominant Th2 pattern and the response to corticosteroids is also described [18], and might be linked to the inhibitory effect of corticosteroids in IL-4, IL-5 and IL-13 gene transcription [24].

Recent independent studies highlight the possible pathogenic role of an imbalance between Th17 cells and Tregs in the pathogenesis of FSGS [17, 25, 26]. Th17 cells induce autoimmune and inflammatory injuries with the production of cytokines such as IL-17, IL-22 and TNFα. Tregs have an anti-inflammatory role and suppress the activation of the immune system by releasing IL-10 and TGF-β1 [4]. Th17 cells and their related cytokines have been found increased and Treg cells decreased in serum of INS patients with relapse in comparison with healthy controls [25, 26]. IL-17 has been found to be increased in kidney biopsies of FSGS patients in comparison with other histological patterns and it has also been positively related with glomerulosclerosis index. It has been hypothesized that this cytokine may promote renal injury-causing alterations in nephrin phosphorylation and apoptosis of podocytes [25]. In our cohort, ∼30% of patients (CL1) showed this Th17-type response pattern.

The third CL (CL3) observed in our is the one that included the higher number of patients and was characterized by increased levels of IL-6, TNFα, Hx, Hgl and suPAR that, taken together, indicate an activation of the acute-phase inflammatory response that was not associated to any defined type of polarization of Th cell subpopulations. IL-1, IL-6 and TNFα are the main cytokines released by monocytes in response to infections or tissue injuries. These cytokines cause a systemic effect by the stimulation of the acute-phase response in the liver [27]. Altogether, the two CLs that were associated with signs of increased activation of the inflammatory response, whether mediated by Th17 cells or by the IL-1/IL-6 pathway (CL1 and CL3), were associated with a similar prevalence of cortico-resistance that, in both cases, was significantly higher than that observed in CL2. These data are in agreement with those described in previous studies in which increased serum of IL-6, Hx and Hgl, have been associated with steroid resistance [28, 29], and with the data from a recent study by our group, showing that increased circulating levels of IL-6, Hx and Hgl were associated with steroid-resistance in patients with MCD [30].

Our data provide a comprehensive view on the pleomorphic immune and/or inflammatory response that can be found at the time of diagnosis in patients with FSGS but they do not allow a direct pathogenic role to be attributed to any of the observed response patterns. Even though there is experimental evidence indicating that both IL-17 and IL-13 can induce injury to podocytes [19, 31], there is no evidence enough to support that these cytokines are directly involved in podocyte injury in human disease. Furthermore, in clinical conditions associated with increased IL-17 levels, such as seronegative spondyloarthropathies, a higher incidence of FSGS has not been described, nor has it been described in patients with chronic rhinosinusitis, asthma or nasal polyposis, in which IL-13 levels are increased.

The main strength of our study is the inclusion of a large homogeneous sample of patients, studied at the time of diagnosis without therapeutic interferences, using a multidimensional marker analysis of the inflammatory and immunological response. When interpreting our results, it must be taken into account that we measured the levels of cytokines and Th cell subtypes in the bloodstream, and this is not necessarily representative of what occurs at the level of the lymphoid organs. The differentiation of Th0 'naïve' lymphocytes into mature Th lymphocytes, takes place at a local level in the lymphoid organs, driven by signals given by the antigen-presenting cells [17, 22, 32]. IL-12 leads to differentiation in Th1 cells, which produce IL-2 and IFNγ, while IL-4, IL-13 and IL-2 lead to Th2 cells, which produce IL-4, IL-13 and IL-5, and the signal induced by IL-6, TGF-β and IL-21 induces differentiation to Th17 cells, which produce IL-17, IL-22, IL-6 and TNFα and exert a powerful pro-inflammatory effect [25, 26]. In our study, ILs like IL-17, IL-13 and IL-4, aggregated into the same CL together with their corresponding synthetizing cells. In other cases, however, the IL and cytokine levels measured in the bloodstream did not concur with that is known to occur locally in the lymphoid organs. This explains the absence of association between IL-12 and IFNγ with Th1 cells and the absence of association between Th17 cells and IL-6.

In conclusion, our data indicate that the patterns of the inflammatory response and Th cell polarization found in patients with FSGS at the time of diagnosis can be classified into three different CLs. The most frequent pattern is consistent with an activated inflammatory response with no polarization of Th cell subtypes. The second pattern in order of frequency is consistent with a predominant Th17-driven response. Finally, the least prevalent pattern has all the characteristics of a Th2-driven response. Patients with a predominant Th2 response showed the best response rate to corticosteroids, while Th17 or IL-1/IL-6 responses were associated with a higher prevalence of cortico-resistance. Together, these data provide useful information for future studies in order to analyse the pathogenic significance of each of the identified response patterns.

SUPPLEMENTARY DATA

Supplementary data are available at ckj online.

CONFLICT OF INTEREST STATEMENT

None declared.

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