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Original Article

ORIGINAL ARTICLE

Activation of the acute inflammatory phase response in idiopathic nephrotic syndrome: association with clinicopathological phenotypes and with response to corticosteroids

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ABSTRACT

Background. Data on the activation of the acute inflammatory response and its clinicopathological associations in idiopathic nephrotic syndrome (INS) are scarce and discordant.

Objective. To analyse the associations between the activation of the inflammatory response, the clinicopathological characteristics of disease and the response to treatment with steroids in patients with INS.

Methods. A total of 101 patients with INS due to minimal change disease (MCD; $n = 44$), focal segmental glomerulosclerosis (FSGS; $n = 33$) and membranous nephropathy (MN; $n = 24$) and 50 healthy controls were included. At diagnosis, we measured the levels of haemopexin (Hx), haptoglobin (Hgl), interleukin-6 (IL-6), soluble urokinase-type plasminogen activator receptor (suPAR), tumour necrosis factor-α (TNF-α), soluble IL-1 receptor, interferon-γ and C-reactive protein. We analysed their clinicopathological associations. In MCD and FSGS patients, we determined the association between the levels of these variables and steroid resistance.

Results. The levels of Hx, Hgl, TNF-α, suPAR and IL-6 were higher in patients with INS than in healthy controls, and were not associated with proteinuria, estimated glomerular filtration rate or serum albumin. In MCD and FSGS patients, Hx, Hgl, IL-6 and TNF-α levels were similar and significantly higher than in MN patients. In patients with MCD and FSGS, multivariate analyses identified FSGS and the levels of Hx, Hgl or IL-6 as independent predictors of steroid resistance.

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Conclusions. The activation of the inflammatory response in patients with INS is heterogeneous and more prevalent in MCD or FSGS patients than in those with MN. In MCD and FSGS, elevated levels of Hx, Hgl or IL-6 are independently associated with steroid resistance.

Keywords: biomarker, glomerulosclerosis, inflammation, minimal change disease, nephrotic syndrome

INTRODUCTION

Minimal change disease (MCD) and focal segmental glomerulosclerosis (FSGS) are two of the most prevalent primary glomerular diseases causing idiopathic nephrotic syndrome (INS) in both children and adults [1, 2]. In both entities, the response to steroids has been identified as the main long-term outcome variable [3, 4]. Although steroid resistance is more frequently associated with the histopathological pattern of FSGS, this association is not invariable [5]. Multiple studies have been carried out with the objective of analysing biomarkers that allow the identification of steroid-resistant patients at diagnosis, but to date, there has been no biomarker that meets this objective [6, 7]. In both diseases, the pathogenesis is unknown and has been linked to the presence of a circulating soluble factor capable of inducing podocyte injury [8–11]. Among them, studies conducted by independent research groups have described a relationship between haemopexin (Hx) and haptoglobin (Hgl), two proteins involved in the transport and metabolism of haemoglobin, and the pathogenesis and/or the response to steroids in patients with MCD or FSGS [12–18]. Hx is a β-1 glycoprotein whose function is to maintain iron homeostasis by binding and transporting the free haem group for liver clearance [19, 20]. An isoform of Hx with protease activity, capable of inducing glomerular injury similar to that observed in MCD in experimental models, has been isolated from human plasma [12–13, 21]. Data available in humans are limited to a single study in which the pathogenic role of Hx in MCD was related to the presence of an isoform with serine protease activity, while total Hx levels were reduced during outbreaks of activity and recovered after remission [22]. In the case of Hgl, two studies have found an association between high serum levels and steroid resistance [17, 18]. The synthesis of Hx and Hgl increases in response to haemolysis [23], but also in response to the stimulation of interleukin-6 (IL-6), and their circulating levels increase significantly as part of the acute inflammatory response [21, 24, 25]. Since an increase in intravascular hemolysis has not been described in nephrotic syndrome, it is conceivable that, as occurs with other molecules such as the soluble urokinase-type plasminogen activator receptor (suPAR) [26], serum levels of Hx and Hgl can vary, depending on the degree of activation of the acute inflammatory response. The acute-phase response is stimulated by the release of cytokines, including IL-1, IL-6 and tumour necrosis factor-α (TNF-α). These cytokines are released mainly by monocytes and cause a systemic effect through stimulation of the acute-phase response by the liver. Studies that analysed the serum level of the main cytokines and acute-phase proteins involved in the inflammatory response in patients with nephrotic syndrome have led to discordant results [27–35]. Some of these studies described an increase in the levels of IL-6, IL-1 and TNF-α [28, 32, 35], whereas others reported that the levels of these cytokines were not higher, compared to healthy controls [27, 31, 33, 34]. To date, there has been no integrated study performed on the activation of the acute-phase inflammatory response in patients with nephrotic syndrome, and no investigation of whether this activation differs depending on the histopathological type of kidney disease and/or whether it has any influence on the clinical profile of the disease. On the other hand, since steroids have been shown to have strong effects on the inflammatory response and are recognized as the first-line treatment for both MCD and FSGS, it seems reasonable to assess whether activation of the inflammatory response has any influence on the response to steroid treatment.

In this study, the levels of IL-6, TNF-α, soluble IL-1 receptor (sIL1R), interferon-γ (IFN-γ), Hx, Hgl, suPAR and C-reactive protein (CRP) were measured at the time of diagnosis in a cohort of patients with INS caused by MCD, FSGS or membranous nephropathy (MN), as well as in healthy controls, with the objectives of: (i) assessing the state of the systemic inflammatory response in patients with nephrotic syndrome of different histopathological types and pathogenesis, (ii) analysing the clinical, biochemical and histopathological characteristics associated with serum levels of proteins related to inflammation and (iii) analysing whether, in patients with MCD or FSGS, activation of the inflammatory response is associated with the response to steroids.

MATERIALS AND METHODS

Patients

This was a cross-sectional observational study performed between 2012 and 2019 at the Nephrology Departments of three tertiary hospitals. We included patients with INS who met all the following inclusion criteria: (i) diagnosis of MCD, FSGS or MN confirmed by renal biopsy, (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. The definitions, diagnostic criteria used and systematic studies performed are detailed in the Supplementary Material section.

All participants provided informed signed consent before study inclusion. The study was performed in accordance with the parameters established by the declaration of Helsinki and the local personal data protection law (LOPD 15/1999). The study protocol was approved by the independent bioethics committee of the participating centres.

Controls

All biochemical reference values were obtained from a control group of 50 age- and sex-matched healthy individuals selected from an electronic database including demographic data and a biobank with serum samples obtained from 450 blood donors who were healthcare professionals and medical students'.

Methods

Serum creatinine levels were measured by a traceable IDMS compensated method (Hitachi Modular P-800 Roche Diagnostics, Manheim, Germany). The estimated glomerular

filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration equation (CKD-EPI) formula in adults and the modified Schwartz equation in children. Hx levels were determined by nephelometry (Coulter Biotek, Berlin, Germany). The levels of Hgl, IL-6, suPAR and sIL1R were measured by enzyme-linked immunosorbent assay (Quantikine R&D Systems, Inc., Minneapolis, MN, USA). The levels of TNF-α and IFN-γ were measured using the MILLIPLEX® MAP system (catalogue number HCYTOMAG-60K Millipore Corporation, city, MO, USA). CRP levels were determined by the Behring Nephelometry immunoassay (NA Latex CRO, Behring Institute, Galway, Ireland). To assess for the reproducibility of the measures, in a sample of 30 patients, three or more determinations were made during the nephrotic outbreak phase prior to the start of corticosteroid treatment. In all cases, the variation coefficients of repeated measures were <13%.

Outcome measure: response criteria to corticosteroid treatment

After diagnosis, both clinical management and follow-up were carried out according to the KDIGO 2020 guidelines. All patients received corticosteroid treatment. Paediatric patients were started on oral prednisone 60 mg/m^2/day for 4 weeks and after remission, they were maintained on prednisone 40 mg/m^2 on alternate days for 2–5 months, with appropriate dose tapering. Adult patients were started on prednisone 1 mg/kg/day (maximum 80 mg) or 2 mg/kg/day (maximum 120 mg) on alternate days for 4 weeks and following remission, the dose was tapered slowly over a period of 6 months. The criteria for complete and partial remission were also defined according to the KDIGO 2020 guidelines [22]. In paediatric patients, steroid resistance was defined as lack of complete remission {reduction of proteinuria urine protein/creatinine ratio (uPCR) <0.2 mg/mg} after 8 weeks of therapy with prednisone at a standard dose of 60 mg/m^2/day. In adult patients, steroid resistance was defined as no remission (reduction of proteinuria <0.3 g/day, stable serum creatinine levels and serum albumin levels >3.5 g/dL) after a minimum exposure of 16 weeks of prednisone at a daily single dose of 1 mg/kg or alternate-day single dose of 2 mg/kg.

Pathological analysis of kidney biopsies

Biopsies were stained with haematoxylin–eosin, periodic acid–Schiff-methenamine and Masson's trichrome for histological analysis. Immunofluorescence studies were carried out using antibodies against immunoglobulin A, immunoglobulin G, immunoglobulin M, C3, fibrinogen and light chains, and analysed by electron microscopy. All biopsies were assessed by the same team of pathologists.

Statistical analysis

Quantitative variables are expressed as the mean ± 1 standard deviation (SD), and qualitative variables as a proportion. Comparisons of the means between groups for independent data were made using the Student's *t*-test in the case of two means, or using the analysis of variance (ANOVA), with Bonferroni correction, in the case of more than two means. Comparison between categorical variables was performed using the Chi-square test.

The association between quantitative variables was analysed using the Pearson correlation coefficient. According to the distribution of variable values in the sample of patients studied,

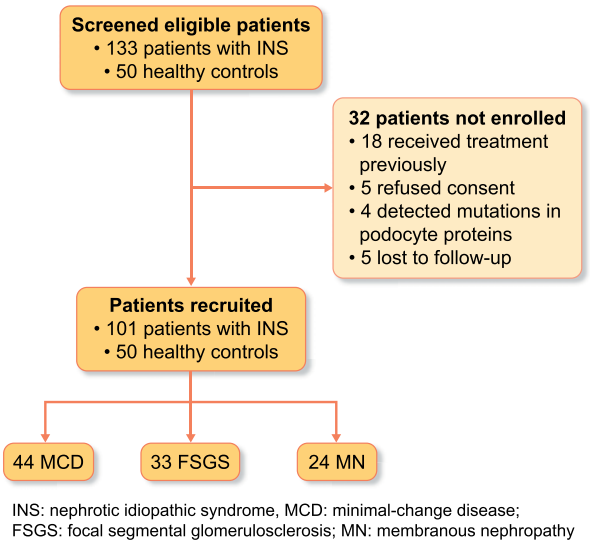

Fig. 1. Flow chart of patient selection.

the sample size included provides a statistical power of 0.84 for Hx, 0.88 for Hgl, 0.85 for TNF-α and IFN-γ, 0.81 for suPAR, 0.85 for IL-6 and 0.82 for sIL1R to determine the differences in means among groups by ANOVA, with an alpha error of 0.05, while excluding outliers from the analysis.

After classifying the patients as having either MCD or FSGS, according to their response to corticosteroid treatment, a univariate analysis was performed to analyse the differences between the two groups. The cut-off values of quantitative variables associated with response to treatment were calculated by receiver operating characteristic (ROC) curves, using the Youden index to select the optimal value [36]. Finally, a step-by-step logistic regression analysis was performed with manual introduction of the variables, to identify variables independently associated with the response to corticosteroid treatment. To compare the logistic models obtained, the integrated discrimination improvement (IDI) index and the net reclassification improvement (NRI) index were calculated [37]. Any P-values <0.05 were considered significant. Statistical calculations were performed using the SPSS program, version 20.0.

RESULTS

We recruited a cohort of 133 patients (53 MCD, 46 FSGS and 34 MN) (Figure 1). From this cohort, we excluded 32 patients for the following reasons: 18 patients received steroids, immunosuppressants, angiotensin II receptor antagonists or statins; 5 patients did not give their written consent; 5 patients were lost to follow-up within the first 3 months after diagnosis; and 4 patients were excluded because they carried known pathogenic mutations in genes encoding proteins of glomerular filtration barrier (NPHS2, $n = 1$; collagen IVα3, $n = 2$; and LMB1X, $n = 1$) (see Supplementary Material). The final study group included 101 patients: 44 MCD, 33 FSGS and 24 MN. Of these patients, 20% were paediatric and 80% were >18 years. Also included were 50 healthy controls.

Table 1. Baseline characteristics of study patients

	MCD (N = 44)	FSGS (N = 33)	MN (N = 24)	Control (N = 50)	P1	P2	P3	P4	P5	P6
Demographic characteristics										
Age (years), mean (SD)	41.7 (19.3)	41.58 (18.7)	45.1 (12.1)	46.4 (20.7)	1	1	1	0.59	0.73	0.65
Male gender, number (%)	27 (61.3)	18 (54.5)	13 (54.1)	27 (54)	0.86	0.86	0.86	0.86	0.86	0.86
Clinical characteristics										
Creatinine (mg/dL), mean (SD)	0.97 (0.3)	1.78 (1.8)	1.32 (0.5)	0.7 (0.3)	**0.02**	0.61	0.754	0.58	**0.023**	**0.046**
eGFR (mL/min/1.73 m^2), mean (SD)	96.3 (22.7)	86.3 (24.4)	85.42 (17.4)	94.1 (16.7)	0.27	0.227	1.000	0.45	0.06	0.07
Biochemical characteristics										
Proteinuria (g/dL), mean (SD)	7.4 (3.2)	6.4 (3.0)	8.0 (3.3)	0.01 (0.1)	0.76	0.87	0.69	**0.000**	**0.000**	**0.000**
Albumin (g/dL), mean (SD)	2.3 (0.5)	2.5 (0.7)	2.5 (0.6)	4.4 (0.2)	0.504	0.688	1.000	**0.000**	**0.000**	**0.000**
CRP (mg/dL), mean (SD)	0.8 (0.6)	0.5 (0.5)	0.2 (0.1)	0.09 (0.03)	0.47	0.29	**0.04**	**0.000**	**0.000**	**0.000**
sIL1R (pg/mL), mean (SD)	1894 (710)	2437 (1375)	1772 (808)	1559 (926)	0.200	1.000	0.126	0.39	0.19	0.34
IL-6 (pg/mL), median (interquartile 25–75)	7.2 (0.9–10.3)	6.9 (0.4–9.8)	1.8 (0.6–2.1)	0 (0–1.6)	1.000	**0.031**	0.043	**0.000**	**0.000**	**0.000**
Hx (mg/dL), mean (SD)	745 (479)	662 (556)	263 (128)	102 (38)	1.000	**0.000**	**0.004**	**0.000**	**0.000**	**0.000**
Hgl (mg/dL), mean (SD)	268.9 (128.6)	228.5 (121.1)	154 (41.2)	98 (51)	0.384	**0.000**	**0.043**	**0.000**	**0.000**	**0.000**
TNF-α (pg/mL), mean (SD)	7.8 (4.3)	8.4 (3.2)	4.7 (3.6)	4.62 (1.2)	1.000	**0.005**	**0.001**	**0.008**	**0.007**	**0.034**
IFN-γ (pg/mL), mean (SD)	15.8 (8.8)	15.1 (9.1)	13.8 (7.2)	16.7 (5.1)	1.000	1.000	1.000	0.66	0.65	0.64
suPAR (ng/mL), mean (SD)	3915 (1216)	4135(1522)	3236 (1055)	3160 (1234)	1.000	0.303	0.097	**0.028**	**0.041**	0.38

P1: MCD versus FSGS; P2: MCD versus MN; P3: FSGS versus MN; P4: MCD versus control; P5 FSGS versus control; P6: MN versus control.
*ANOVA test followed by between-group comparisons with Bonferroni correction.
The bold values are the significant p ($p<0.04$).

Clinical and biochemical characteristics

Table 1 summarizes the demographic, clinical and biochemical characteristics of the three groups of patients with nephrotic syndrome and the healthy control group. No differences in age, sex, eGFR, serum albumin or proteinuria were observed among the three groups of nephrotic syndrome patients. Patients with FSGS had higher CRP levels compared with those with MN. IL-6 levels were significantly higher in patients with MCD than in MN patients. In comparison with MN patients, the levels of Hx, Hgl and TNF-α were higher in patients with MCD and FSGS, with no significant differences between the MCD and FSGS groups. In the three groups of nephrotic syndrome patients, the levels of Hx, Hgl, TNF-α and IL-6 were significantly higher than in healthy controls. suPAR levels were lower in healthy controls, compared with patients with FSGS and MCD. The levels of sIL1R and IFN-γ were similar in all kidney disease groups and showed no significant differences when compared with the healthy controls. Figure 2 shows the distribution of the different biochemical parameters analysed in the three groups of nephrotic syndrome patients and in healthy controls.

The associations observed between the variables studied were different in each type of glomerular disease (see Tables 2–4).

In patients with MCD, the levels of Hx, Hgl, IL-6 and suPAR were significantly associated with each other. CRP levels were associated with Hx, Hgl and IL-6 levels. The levels of sIL1R were associated with eGFR, and TNF-α and suPAR levels showed an association with proteinuria. IFN-γ levels did not show associations with any of the variables analysed.

In FSGS patients, the levels of Hx, Hgl, CRP and IL-6 were associated with each other. Hx levels were associated with suPAR levels, and inversely associated with IFN-γ levels. sIL1R levels were associated with eGFR. TNF-α levels did not show associations with any of the variables analysed.

In MN patients, the levels of Hx and Hgl were associated with each other, but not with the levels of IL-6 or suPAR. Hgl levels were associated with TNF-α levels. The levels of sIL1R were associated with both eGFR and suPAR levels. IFN-γ showed no significant association with any of the variables analysed.

Variables associated with response to steroid treatment in patients with MCD or FSGS

Overall, 27/77 patients (35%) showed steroid resistance. In the univariate analysis, the variables associated with steroid resistance were: histopathological pattern of glomerular disease (FSGS 17/33, 51.5% versus MCD 10/44, 22.7%; P = 0.008) and the levels of albumin, IL-6, Hx and Hgl (Table 5). Levels of CRP, TNF-α, suPAR, IFN-γ and sIL1R were not associated with corticosteroid resistance. Using receiver operating characteristic curves (ROC curves), the values with the best combination of sensitivity and specificity to predict steroid resistance, according to the Youden index, were ≥112.5 pg/mL for Hx, ≥257.9 mg/dL for Hgl and ≥11.6 pg/mL for IL-6. The final logistic regression model obtained after a forward selection of variables included FSGS and either Hx, Hgl or IL-6 as independent predictors of steroid resistance. When added to FSGS in the logistic models, each of these three biomarkers showed a similar statistical significance as predictors of steroid resistance. Table 6 summarizes the area under the curve (AUC), IDI and NRI of the logistic models obtained with each variable. Since the association between the levels of Hx, Hgl and IL-6 and steroid resistance was not linear, the levels of these three variables were introduced in the logistic models after categorizing them into two categories according to the values of the cut-off points previously obtained from the ROC curves.

DISCUSSION

In this cross-sectional study, we measured, at the time of diagnosis, the serum levels of the main cytokines involved in the stimulation of the acute inflammatory response and the levels of Hx, Hgl, suPAR and CRP, as representative of acute-phase

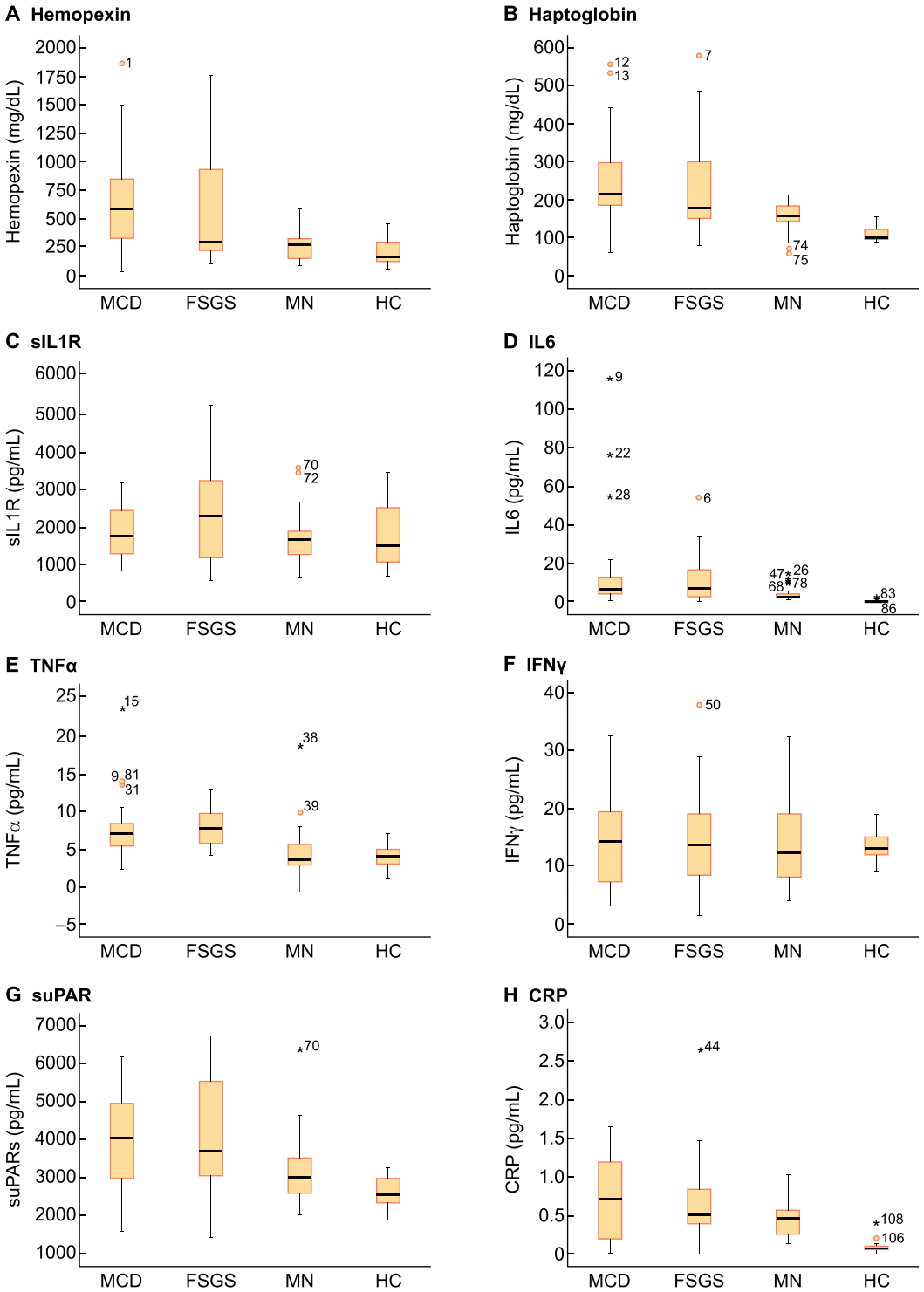

Fig. 2. Box plot diagrams of the serum levels of Hx, Hgl and inflammatory biomarkers in the four subgroups of analysis: MCD, FSGS, MN and controls. (**A**) Hx. (**B**) Hgl. (**C**) sIL1R. (**D**) IL-6. (**E**) TNF-α. (**F**) IFN-γ. (**G**) suPAR. (**H**) CRP. Outliers and extreme outliers are represented.

response proteins, in a cohort of patients with INS caused by different pathogenic mechanisms. Our study has shown a number of important results. The first finding of interest is, when compared with healthy controls, the three groups of patients with nephrotic syndrome showed comparable levels of sIL1R and IFN-γ, but significantly increased levels of IL-6, TNF-α, Hx, Hgl and CRP. MCD and FSGS patients showed significantly higher suPAR levels than healthy controls. Our data differ from those described in the only previous study available on the levels of Hx, which showed no elevated Hx levels in any disease causing nephrotic syndrome [24]. The results described in previous studies are also discordant with respect to TNF-α levels. Some studies described an increase in TNF-α levels in patients with active nephrotic syndrome [28, 32, 33, 35], whereas others

Table 2. Correlation matrix among variables in MCD patients

	Age	Creatinine	eGFR	Albumin	Proteinuria	suPAR	Hgl	Hx	TNF-α	IFN-γ	sIL1R	IL-6
Creatinine	0.32											
eGFR	−0.84**	−0.76**										
Albumin	0.01	−0.03	0.06									
Proteinuria	0.07	0.02	−0.01	0.33								
suPAR	0.38*	0.22	−0.38*	−0.26	0.38*							
Hgl	0.05	0.08	−0.06	−0.39*	0.25	0.40*						
Hx	−0.09	−0.01	0.07	−0.42*	0.33	0.49**	0.82**					
TNF-α	0.07	−0.01	−0.037	−0.19	0.40*	−0.26	0.02	0.09				
IFN-γ	−0.07	−0.04	0.018	−0.10	−0.35	−0.18	−0.11	0.05	0.18			
sIL1R	0.38	0.49**	−0.54	0.14	−0.09	0.27	0.19	0.09	−0.10	−0.01		
IL-6	−0.35	−0.28	0.46	0.07	0.15	−0.09	0.45*	0.49**	0.10	−0.01	−0.33	
CRP	0.09	0.07	0.04	−0.16	0.19	0.13	0.36*	0.35*	0.17	0.04	0.28	0.41*

*P < 0.05;
**P < 0.01.

Table 3. Correlation matrix among variables in FSGS patients

	Age	Creatinine	eGFR	Albumin	Proteinuria	suPAR	Hgl	Hx	TNF-α	IFN-γ	sIL1R	IL-6
Creatinine	−0.11											
eGFR	−0.53**	−0.61**										
Albumin	−0.06	0.29	−0.21									
Proteinuria	0.02	−0.16	0.26	−0.23								
suPAR	−0.04	0.36	−0.21	−0.26	0.18							
Hgl	0.26	−0.09	−0.10	−0.40*	0.21	0.31						
Hx	0.27	−0.19	0.01	−0.43*	0.27	0.43*	0.86**					
TNF-α	−0.05	−0.01	−0.06	−0.20	0.03	0.08	0.07	−0.03				
IFN-γ	0.12	−0.08	−0.10	−0.13	−0.09	−0.09	−0.36	−0.38*	−0.15			
sIL1R	0.23	0.58**	−0.54*	0.024	−0.17	−0.11	−0.02	−0.21	0.35	0.28		
IL-6	0.15	−0.16	0.03	−0.27	0.11	0.35	0.70**	0.79**	−0.13	−0.39*	−0.16	
CRP	0.12	0.04	0.07	−0.29	0.16	0.12	0.34*	0.39*	0.09	−0.08	0.14	0.40*

*P < 0.05;
**P < 0.01.

Table 4. Correlation matrix among variables in MN patients

	Age	Creatinine	eGFR	Albumin	Proteinuria	suPAR	Hgl	Hx	TNF-α	IFN-γ	sIL1R	IL-6
Creatinine	0.19											
eGFR	−0.57**	−0.87**										
Albumin	−0.04	0.24	−0.31									
Proteinuria	0.33	−0.11	−0.12	−0.34								
suPAR	0.38	0.39	−0.42	−0.16	−0.06							
Hgl	−0.11	−0.19	0.20	−0.19	0.39	0.05						
Hx	−0.22	−0.16	0.38	−0.23	0.11	0.16	0.47*					
TNF-α	−0.03	−0.18	0.17	−0.42*	0.24	0.53*	0.57**	0.29				
IFN-γ	0.25	−0.07	−0.07	−0.15	0.09	0.08	0.33	0.03	−0.03			
sIL1R	0.36	0.35	−0.55*	−0.42*	−0.07	0.55*	−0.04	−0.39	0.11	0.11		
IL-6	0.29	0.05	−0.23	−0.37	−0.09	0.11	−0.26	−0.38	−0.02	−0.22	0.16	
CRP	0.09	0.03	−0.04	−0.14	−0.06	0.03	0.12	0.13	0.05	0.06	0.09	0.11

*P < 0.05;
**P < 0.01.

found no differences compared with healthy controls [18, 26, 30]. Published results on IL-6 levels have also been discordant [34]. The disagreement among studies could be partly explained by the interference of external variables related to drug exposure at the time when measurements were performed, to the inclusion of patients with different stages of disease activity, to different criteria used for histopathological classification, and to technical aspects related to the methods of measurement. Moreover, it must be taken into account that the systemic inflammatory response is triggered by the production of proinflammatory cytokines and is sequentially orchestrated [38]. The first cytokines synthesized are IL-1 and TNF-α, which, in a second stage, stimulate the production of IL-6. The synthesis of acute-phase proteins takes place in the liver, after stimulation by these cytokines. Some acute-phase proteins (Hgl, Hx, suPAR or CRP) are more sensitive to IL-1 stimulation, whereas others (fibrinogen and α-1 antitrypsin) are more sensitive to IL-6 stimulation. Therefore, the profile of cytokines and circulating acute-phase proteins observed in the bloodstream depends on both the persistence of proinflammatory stimulation and the time interval between the onset of inflammatory stimulation and the time of measurements. The actual timing and stage of the acute phase relative to the time of sample collection could partly account for the different profiles of cytokines and acute-phase proteins observed in patients showing a stimulated acute inflammatory response. Despite their increased mean levels, the serum levels of IL-6, TNF-α, Hx, Hgl, suPAR and CRP showed high interindividual variability within the three groups of patients analysed, ranging from values comparable to those of healthy controls to extremely high values. This variability indicates that only a subset of patients with nephrotic syndrome have high levels of these inflammatory proteins. When analysing the variables associated with the levels of Hx, Hgl, TNF-α, suPAR and IL-6, a significant association with each other was observed, and in all cases, their levels were independent of age, sex, eGFR or the severity of nephrotic syndrome, assessed in terms of urinary protein excretion and serum albumin levels. We found significant differences in the levels of inflammatory proteins among the three groups of patients with nephrotic syndrome, since the levels of Hx, Hgl, TNF-α and IL-6 were similar in patients with MCD and FSGS and, in both the MCD and FSGS groups, the levels were significantly higher than in patients with MN. These data indicate that, at the time of diagnosis, activation of the inflammatory response was more prevalent in MCD and FSGS than in MN, either in relation to a different pathogenic mechanism or in relation to possible triggers not necessarily related to the pathogenesis. The interindividual variability of Hx, Hgl, TNF-α and IL-6 levels in patients with MCD and FSGS was not related to the severity of proteinuria, which rules out a pathogenic link between activation of the acute inflammatory response and the pathogenesis of both diseases that could be common to all patients. However, it does not rule out that activation of the acute inflammatory response can play a precipitating or pathogenic role in a certain subgroup of patients.

The most relevant result of our study showed a significant association between elevated levels of Hx, Hgl and IL-6 and steroid resistance in patients with MCD and FSGS. This relationship is not linear and is only significant for high levels of these variables. The histopathological pattern of glomerular disease was the best predictor of steroid resistance and gives a likelihood of steroid resistance of 51.5% in patients with FSGS and 22.7% in patients with MCD. The predictive capacity of the models, however, improves significantly and similarly when the levels of Hx or Hgl or IL-6 are combined with the histopathological

Table 5. Baseline characteristics of MCD and FSGS patients with or without steroid resistance

Variables	SS ($n=50$)	SR ($n=27$)	P
Age (years)	39.8 ± 19.2	45.3 ± 18.2	0.22
Creatinine (mg/dL)	1.35 ± 1.5	1.10 ± 0.4	0.40
eGFR (mL/min/1.73 m^2)	92.8 ± 23.9	87.3 ± 24	0.46
Proteinuria (g/d)	10.1 ± 3.8	11.2 ± 2.7	0.78
Albumin (g/dL)	2.6 ± 0.6	2.4 ± 0.5	**0.003**
sIL1R (pg/mL)	2122.9 ± 1110	2133.9 ± 976.9	0.97
IL-6 (pg/mL)	5.6 (0.6–9.1)	9.4 (0.3–23.5)	**0.03**
Hx (mg/dL)	549 ± 474	1061 ± 417	**0.000**
Hgl (mg/dL)	208.7 ± 101.1	344.1 ± 127.8	**0.000**
TNF-α (pg/mL)	7.8 ± 3.4	8.7 ± 4.8	0.35
IFN-γ (pg/mL)	16.2 ± 9.1	13.9 ± 8.4	0.31
suPAR (ng/mL)	3856.9 ± 1312.1	4536.3 ± 1455.1	0.10
CRP (mg/dL)	0.82 (0.71)	0.96 (0.64)	0.49

*Data are presented as mean ± SD of median.
Data are presented as mean ± SD of median.
The bold values are the significant p ($p<0.04$)

Table 6. Logistic regression models to predict corticosteroid resistance and IDI and NRI indices using Hx, Hgl and IL-6, in addition to pathology variables

Variables	B	SE	OR (95% CI)	Sig.	AUC	P	IDI	P	NRI total	P	NRI R	P	NRI S	P
Model with pathology														
FSGS (1)	1.4	0.5	4.2 (1.5–11.5)	0.005	0.68 (0.06)	0.000								
Model with pathology + Hx levels														
FSGS (1)	2.1	0.9	8.2 (1.3–50.3)	0.022	0.78* (0.06)	0.000	0.17 ± 0.05	0.02	44.7 ± 0.2	0.033	40.7 ± 0.18	0.03	4 ± 0.09	0.8
Hx	1.5	0.6	7 (1.5–32.5)	0.012										
Model with pathology + Hgl levels														
FSGS (1)	1.8	0.8	6.2 (1.2–34.1)	036	0.76* (0.57)	0.000	0.16 ± 0.06	0.03	43.1 ± 0.2	0.038	37.5 ± 0.19	0.04	6 ± 0.10	0.54
Hgl	1.5	0.6	4.2 (1.3–14)	0.023										
Model with pathology + IL-6 levels														
FSGS (1)	1.8	0.7	6.3 (1.6–25.4)	0.009	0.73* (0.58)	0.000	0.15 ± 0.03	0.04	51.6 ± 0.2	0.009	29.6 ± 0.17	0.08	22 ± 0.09	0.02
IL-6	1.4	0.6	4.1 (1.2–13.7)	0.024										

IDI and NRI data are presented as mean ± SD.
OR: odds ratio; CI: confidence interval; NRI total: for all patients; NRI R: for corticosteroid-resistant patients; NRI S: for corticosteroid-sensitive patients).
*P = 0.00 versus pathology model.

pattern of glomerular disease. The introduction of each of these variables in the logistic models results in a significant improvement in the IDI and NRI indices, in terms of increasing the likelihood of steroid resistance assigned to those patients who are actually steroid-resistant. The association between Hx levels and steroid resistance had not been previously described. However, two previous studies reported an association between steroid resistance and high levels of Hgl [16, 17], while another study described an association between steroid resistance and elevated levels of IL-6 [39]. Given the significant associations observed among Hx, Hgl and IL-6, their role as biomarkers associated with steroid resistance could be related to the intensity, persistence or dysregulation of the acute-phase inflammatory response. However, steroid resistance is also observed in patients with normal or low levels of Hx, Hgl and IL-6, which indicates that increased levels of these inflammatory markers are only associated with steroid resistance in a certain group of patients. In other cases, steroid resistance is clearly unrelated to an activated inflammatory response.

The main strengths of our study are the inclusion of a large sample of patients, studied at the time of diagnosis and without therapeutic interventions that can cause external interferences in the levels of the variables studied. Similarly, a comprehensive analysis of the inflammatory response was performed. On the other hand, repeated measurements of the analysed variables were made, ensuring reproducibility of the values obtained. Our study has limitations that must be highlighted. First, our data lack external validation and, consequently, can only be considered valid for the group of patients included in the study. Second, the criteria for conducting a genetic study were based on age and response to corticosteroids, and were limited to the number of genes currently known. Consequently, it cannot be ruled out that in the future, with increasing knowledge of the genes involved in these kidney diseases, there would be evidence that some of the patients included in the study carried mutations that were unknown at the time when the study was conducted.

In conclusion, our results indicate that the activation of the acute inflammatory response in patients with nephrotic syndrome is complex, heterogeneous and unrelated to the severity of the disease at the time of diagnosis. While there is a group of patients showing increased levels of TNF-α, IL-6, Hx, Hgl, CRP and suPAR, but normal levels of sIL1R or IFN-γ, other patients with similar levels of proteinuria and hypoalbuminaemia show no evidence of acute inflammatory response activation. Activation of the acute inflammatory response is more prevalent in patients with MCD or FSGS than in those with MN. In patients with MCD or FSGS, elevated levels of Hx, Hgl or IL-6 are independently associated with steroid resistance and improve the predictive value given by the histopathological pattern of FSGS alone. Given the potential relevance of these findings, we believe it is necessary to conduct studies to define, in greater detail, the role of the activation of the inflammatory response in the pathogenesis of both nephropathies, as well as external validation studies to analyse their clinical value as potential predictors of steroid resistance.

SUPPLEMENTARY DATA

Supplementary data are available at ckj online.

CONFLICT OF INTEREST STATEMENT

None declared.

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