www.nephropathol.com DOI: 10.34172/jnp.2021.16 J Nephropathol. 2021;10(2):e16

Journal of Nephropathology

Relevance of glomerular C4d deposition in pediatric patients with Henoch-Schönlein Purpura compared to IgA nephritis

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ARTICLE INFO

Article type:
Original Article

Article history:
Received: 19 November 2020
Accepted: 12 December 2020
Published online: 16 December 2020

Keywords:
C4d staining
Glomerulonephritis
Henoch-Schönlein purpura
IgA nephritis
IgA vasculitis
Pediatric nephrology

ABSTRACT

Introduction: IgA nephropathy (IgAN) is the most common primary glomerulonephritis (GN) in western countries and Henoch-Schönlein purpura nephritis (HSPN) is the most common form of vasculitis in childhood. Renal biopsy findings in both nephropathies are often similar and are characterized by mesangioproliferative GN with mesangial or mesangiocapillary IgA and C3c deposits.

Objectives: The aim of this study was to investigate the significance of glomerular C4d-deposition as a discriminating factor between pediatric HSPN and IgAN.

Patients and Methods: We retrospectively analyzed patient records and renal biopsies from 53 pediatric patients from one single center with a median age of 10.5 years (range 2.3-18 years). Twenty-two patients suffered from IgAN and 31 from HSPN. Work-up of all renal biopsies was performed using standard protocols including immunohistochemistry for C4d.

Results: Pediatric IgAN patients presented significantly more often with gross hematuria, higher serum creatinine, lower glomerular filtration rate, lower serum C3 and proteinuria and on histology less endocapillary hypercellularity compared to HSPN patients. However, the rate of glomerular C4d-positivity was not different between IgAN (36%) and HSPN (42%). Comparing all cases with positive versus negative glomerular C4d-staining, pediatric patients with glomerular C4d-positivity showed significantly lesser gross hematuria and received significantly more often cyclophosphamide. This was in line with a tendency towards more proteinuria, hypertension and renal insufficiency at last follow-up in C4d-positive compared to C4d-negative patients.

Conclusion: In conclusion, in our monocentric study glomerular C4d does not differ between pediatric HSPN and IgAN, but was associated with a tendency to a more severe course of the disease that needs to be confirmed in larger multicentric studies.

Implication for health policy/practice/research/medical education:
The aim of this study was to investigate the significance of glomerular C4d-deposition as a potential discriminating factor between glomerular disease in pediatric Henoch-Schönlein purpura nephritis (HSPN) and IgA nephropathy (IgAN). However, in our monocentric study glomerular C4d does not differ between pediatric HSPN and IgAN, but was associated with a tendency to a more severe course of the disease that might be of potential relevance for the intensity of treatment strategies in some individual patients; however this finding needs to be confirmed in larger multicentric studies.

Please cite this paper as: Benz K, Ferrazzi F, Galiano M, Sauerstein K, Vonbrunn E, Daniel C, Büttner-Herold M, Amann K. Relevance of glomerular C4d deposition in pediatric patients with Henoch-Schönlein Purpura compared to IgA nephritis. J Nephropathol. 2021;10(2):e16. DOI: 10.34172/jnp.2021.16.

Introduction

IgA nephropathy (IgAN) is the most common primary glomerulonephritis (GN) in western countries and Henoch-Schönlein purpura nephritis (HSPN) is the most common form of vasculitis in childhood (1,2). Renal involvement in IgAN and HSPN is often morphologically indistinguishable, so that the differentiation is based solely on clinical findings, as in HSPN cutaneous involvement

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Original Article

usually as purpuric rash is defining. Accordingly, when a patient shows biopsy proven IgAN in the presence of purpura or additional clinical findings such as other organ involvements, the clinical diagnosis is HSPN instead of IgAN. Of note, in the recent Chapel Hill classification HSPN was officially renamed IgA-vasculitis (2) due to its potential presentation with microangiopathy.

Complement factor 4d (C4d) is a degradation product of the complement cascade which is generated during activation of the classical and lectin pathways. In renal transplantation pathology, C4d is routinely used as a marker for active antibody mediated rejection (ABMR) (3). Specifically, C4d-positivity of peritubular capillaries is a strong indicator of ABMR in AB0- compatible renal transplantation. Moreover, specific glomerular C4d-deposition was identified as a prognostic marker in adult and pediatric patients with IgAN but not with HSPN. Positive C4d-staining was seen in 38%-68% of adult patients with IgAN, mostly in a mesangial pattern (4-6). In these studies, patients with glomerular C4d-positivity were older, more frequently presented with hypertension, lower gross hematuria, more proteinuria, more glomerulosclerosis and interstitial fibrosis and lower renal survival than C4d-negative patients pointing to an inferior outcome of C4d-positive IgAN (4). Furthermore, glomerular C4d-positivity was shown to be an independent risk factor for development of end-stage renal disease (ESRD) in adult IgAN patients (5). In pediatric IgAN, C4d-positivity was detected in 21% of cases and was also identified as an independent predictor of worse renal outcome (7).

Most of the above studies, however, excluded patients with HSPN from the analyses. Only Espinosa et al described C4d-positivity in 2/8 adult HSPN patients (4) but there is no information on its correlation to morphology or prognosis. Apart from this report no other studies on glomerular C4d in HSPN in general or in pediatric HSPN have been published. Thus, there is currently no information available whether glomerular C4d-deposition differs between HSPN and IgAN in pediatric patients and might therefore be used as a discriminating factor on renal tissue or whether it might be useful for prognostic or therapeutic purposes.

Objectives

It was the aim of our study to retrospectively analyse renal biopsies of pediatric patients with HSPN and IgAN from one single pediatric nephrology centre with (i) respect to glomerular C4d-staining and (ii) other morphological and clinical findings. Additionally, we were interested in whether glomerular C4d-staining correlates with any of the other findings and might be used to sub-classify the cases or to predict prognosis.

Patients and Methods

Patient selection

All consecutive renal biopsies during a period of 16 years (2002-2018) of patients with biopsy-proven IgAN diagnosed at one single pediatric nephrology unit were analysed. Based on their clinical presentation these patients were divided into 2 groups, i.e. IgAN and HSPN, with the characteristical purpura before or at onset of nephritis as criteria for HSPN. Indication for renal biopsy included persistent (>4 weeks) or recurrent (>3 relapses) gross hematuria, nephrotic range proteinuria or renal insufficiency. Standard diagnostic criteria for histological IgAN or HSPN were used i.e. mesangioproliferation, the presence of predominant granular mesangial (or mesangiocapillary) deposition of IgA and less pronounced C3c in immunohistology, and predominant mesangial (and subendothelial) osmiophilic deposits in electron microscopy. We excluded only one patient with complex juvenile polyarthritis who was treated for several years with different immunosuppressive agents before IgAN was diagnosed. Furthermore, repeat renal biopsies performed in a subgroup of patients with severe renal injury after completion of cyclophosphamide treatment were excluded. The following parameters were documented at the time of renal biopsy and at last follow-up: age (years), gender, body weight (kg), body height (cm), blood pressure (mm Hg), history of gross hematuria, creatinine (mg/dL), C3-complement (g/L), serum protein (g/L), serum albumin (g/L), urine protein in spot urine (mg/g creatinine0, urine protein in 24-hour urine (mg/m^2/d). The minimal required follow-up time after renal biopsy was 6 months. Accordingly, due to the transfer to another hospital 11 of the 53 patients had no adequate follow-up examination.

All renal biopsies were re-evaluated by a renal pathologist (K.A.) in a blinded manner. The following biopsy characteristics were documented; number of glomeruli, mesangial hypercellularity (score 0-2 with 0: absent, 1: mild and 2: moderate), mesangial matrix expansion (yes/no), segmental glomerulosclerosis (yes/no), endocapillary hypercellularity (yes/no), crescents (yes/no), interstitial fibrosis and tubular atrophy (IFTA, %), arterial wall thickening (yes/no) and Oxford classification for IgAN (8-10). In addition, patient records were reviewed for immunosuppressive (steroids, cyclophosphamide and others) and ACE-inhibitor (ACE-i) treatment.

Definitions

Nephrotic-range proteinuria was defined as urinary total protein >2.0 g/g creatinine in spot urine or >1.0 g/m^2/day in 24-hour urine. Non-nephrotic-range proteinuria was defined as urinary loss of protein between 0.2 and 2.0 g/g creatinine or between 0.15 and 1.0 g/m^2/day in

24-hoururine, respectively. The glomerular filtration rate (GFR) was calculated using the Schwartz formula; GFR = height [cm] * 0.413/creatinine [mg/dL] (11). Impaired renal function was defined as a GFR <90 mL/min/1.73 m^2. Hypertension was defined as systolic blood pressure >95th percentile (12).

Immunostaining

Standard protocols and polyclonal antibodies against C4d (rabbit anti-C4d, Zytomed Systems, 1:500), IgA (rabbit anti-human IgA, DAKO Deutschland, 1:150000), IgG (rabbit anti-human IgG, DAKO Deutschland, 1:100000), IgM (rabbit anti-human IgM, DAKO Deutschland, 1:750000), C1q (rabbit anti-human C1q, DAKO Deutschland, 1:750000) and C3c (rabbit anti-human C3c, DAKO Deutschland, 1:75000) were used. Staining was performed using a Ventana BenchMark stainer with pretreatment (ULTRA CC1, Roche Co.). Renal biopsies were semiquantitatively screened for granular mesangial C4d staining (score 0-3 with 0: negative, 1: mild, 2: moderate, 3: strong positivity for C4d), representative immunohistological pictures are shown in figure 2C-F.

Ethical issues

The research followed the tenets of the Declaration of Helsinki. Inform consent was obtained from all patients' parents at the time of biopsy. The study was conducted according to the general guidelines for studies using human material. The use of archival biopsy material was approved by the local ethics committee (Ref # 4415).

Statistical analysis

First, statistical differences between IgAN and HSPN patients were assessed. In case of binary variables, Fisher's exact test (two-tailed) was employed to assess the statistical significance of the association between the IgAN/HSPN diagnosis and the measured binary variable; for all other variables Wilcoxon rank sum test (with normal approximation) was employed to compare both groups. Afterwards, differences between C4d-positive and C4d-negative patients were assessed employing an analogous procedure. All statistical analyses were performed in the R (v. 3.6.1) environment [R Core Team (2019). (R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria; https://www.R-project.org/]).

Results

Patient characteristics

We analysed 53 Caucasian patients, 22 (14 male, 8 female) with IgAN and 31 (15 male, 16 female) with HSPN. The median patient age was 10.5 years with an age range from 2.3 to 18 years.

Laboratory findings, renal biopsy results and treatment of IgAN and HSPN

IgAN was associated with a higher incidence of gross hematuria and a lower extent of proteinuria and endocapillary hypercellularity, but similar C4d-deposition compared to HSPN.

Patients with IgAN were on average about 2 years older than HSPN patients (12.24 ± 3.68 years versus 9.91 ± 4.11 years, P = N.S.) and suffered significantly more often from gross hematuria (72.7% versus 43.3%, $P < 0.05$). Indication for renal biopsy in IgAN patients was recurrent or persistent gross hematuria in 64%, proteinuria in 41% and renal insufficiency in 23%. In contrast, in HSPN patients the most common indication for renal biopsy was proteinuria (81%) and less frequently recurrent or persistent gross hematuria (26%) or renal insufficiency (3%), respectively. Of course, in both groups more than one biopsy indication was observed in some individuals. At the time of renal biopsy, 23% of IgAN and 32% of HSPN patients had arterial hypertension (P = N.S.). Serum creatinine levels were significantly higher in IgAN compared to HSPN with more IgAN patients showing renal insufficiency as indication for renal biopsy. In line with these results, glomerular filtration rate (GFR) was significantly lower in IgAN compared to HSPN patients (101.5 ± 41.8 mL/min/1.73 m^2 and 120.4 ± 33.0 mL/min/1.73 m^2; $P < 0.05$). Proteinuria was extremely variable in both groups and ranged from no to massive nephrotic range proteinuria. However, proteinuria (24-hour urine as well as spot urine) was significantly ($P < 0.05$) higher in HSPN compared to IgAN patients (Table 1). Significantly more HSPN than IgAN patients showed nephrotic range proteinuria (58% versus 23%, $P < 0.05$) and significantly less had no proteinuria at the time of biopsy (13% versus 23%, $P < 0.05$). In parallel, serum protein and albumin values were significantly lower ($P < 0.05$ and $P < 0.005$, respectively) in HSPN compared to IgAN (Table 1). Interestingly, mean serum C3 levels were in the normal range in both groups but showed significantly higher values in HSPN compared to IgAN patients (1.28 ± 0.17 g/L versus 1.15 ± 0.16 g/L, $P < 0.05$).

On renal histology, there was no significant difference between both groups with respect to percentage of global or segmental glomerulosclerosis or crescent formation (52% in HSPN versus 36% in IgAN, P = N.S.; Figure 1A-D). Both groups showed typical glomerular staining for IgA (Figure 1E and F) and C3c (Figure 1G, H). Of particular note, the percentage of endocapillary hypercellularity (Figure 1A-D) was significantly higher in HSPN compared to the IgAN (52% versus 18%; $P < 0.05$). Positive glomerular C4d-staining was found in a similar percentage in both groups, i.e. in 36% of IgAN

Table 1. Comparison of pediatric patients with IgAN and HSPN

	IgAN (n=22)	HSPN (n=31)	*P* value
Age [years]	13.25 (6-17.5)	9.75 (2.25-18)	0.054
Gender [male/female]	14/8	15/16	ns
History of gross hematuria [% of patients]	72.7%	43.3%	<0.05
Hypertension [% of patients]	22.7%	32.3%	ns
Laboratory findings			
Creatinine [mg/dL]	0.60 (0.32-11.27)	0.44 (0.23-1.75)	<0.05
GFR [mL/min/1.73m^2]	110.8 (5.72-176.8)	124.8 (33.0-170.5)	<0.05
Serum protein [g/L]	68 (54-80)	63 (48-83)	<0.05
Serum albumin [g/L]	41 (29-48)	34 (19-44)	<0.005
Serum C3 [g/L]	1.17 (0.72-1.34)	1.28 (1.01-1.7)	<0.05
Protein in spot urine [mg/g creatinine]	891 (107-7590)	2157 (129-23595)	<0.05
Protein in 24-h urine [mg/m^2/d]	467.5 (49-3700)	1140 (57-5980)	<0.05
Renal biopsy results			
Glomerulosclerosis [% of patients]	18.2%	19.4%	ns
Segmental sclerosis [% of patients]	59.1%	58.1%	ns
Endocapillary hypercellularity [% of patients]	18.2%	51.6%	<0.05
Crescents [% of patients]	36.4%	51.6%	ns
C4d positivity [% of patients]	36.4%	41.9%	ns
Last follow up			
Time span biopsy to last follow up [months]	37.5 (9-118)	43 (6-115)	ns
Last follow up: GFR <90 mL/min/1.73 m^2 [% of patients]	20% (3/15)	18.5% (5/27)	ns
Last follow up: presence of proteinuria [% of patients]	7.1 % (1/14)	18.5% (5/27)	ns
Treatment			
Immunosuppressive treatment [% of patients]	47.6%	66.7%	ns
Cyclophosphamide treatment [% of patients]	19%	20%	ns
ACE-inhibitor treatment [% of patients]	71.4%	96.7%	<0.05

GFR, glomerular filtration rate; Data shown as median (range), ns: $P > 0.05$.

and 42% of HSPN (P=N.S.). More detailed evaluation of glomerular C4d-staining using a semi-quantitative scoring system (0-3) showed neither differences in mean values of IgAN and HSPN (Figure 2A), nor in distribution of score values (Figure 2B). The Oxford classification which should formally not be used for HSPN showed a significantly higher number of Oxford E1 class in HSPN patients which is in accordance with the higher rate of endocapillary hypercellularity. All other classification criteria were not different between both groups (data not shown). In further follow-up, 48% of IgAN and 67% of HSPN patients received at least one immunosuppressive drug (P=N.S.). The use of ACE-i was significantly ($P<0.05$) lower in IgAN (71%) than in HSPN patients (97%). At last follow-up which was at a median of 38 months (IgAN) and 43 months (HSPN) a similar amount of patients, i.e. 20% in IgAN and 19% in HSPN, showed reduced GFR <90 mL/min/1.73 m^2. Interestingly, a slightly higher number of HSPN patients (19%) showed proteinuria compared to IgAN (7%) (P=N.S.).

Laboratory findings, renal biopsy results and treatment in C4d-positive versus C4d-negative IgAN and HSPN patients

Glomerular C4d-positivity was associated with lower incidence of gross hematuria and more cyclophosphamide treatment

Out of the 53 patients, 21 showed positive and 32 negative glomerular C4d-staining with no significant difference between IgAN and HSPN (C4d+: IgAN/HSPN: 8/13, C4d-: IgAN/HSPN: 14/18). Gross hematuria was significantly more frequent in C4d-negative patients (68% versus 38%, $P<0.05$) and cyclophosphamide treatment was less common (9.7 versus 35%, $P<0.05$) than in C4d-positive patients. Hypertension (43% versus 19%, P=N.S.) tended to be more common and 24 h proteinuria was somewhat higher (1082 versus 695 mg/m^2/d, P=N.S.) in C4d-positive compared to C4d-negative patients (Table 2). Renal function, determined by serum creatinine and GFR, and mean serum C3 levels did not significantly differ between the groups. Indication for renal biopsy slightly varied between C4d-positive and

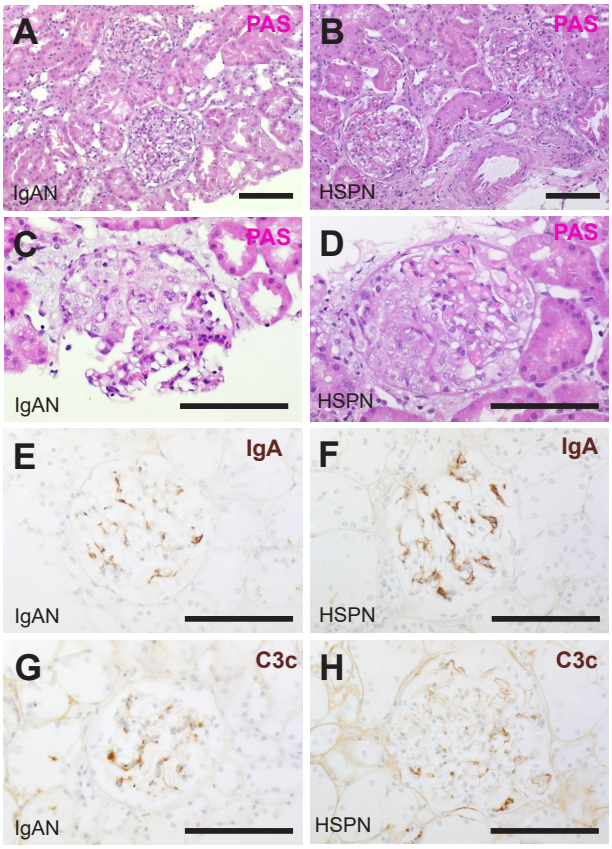

Figure 1. Representative morphological and immunohistochemical findings in pediatric IgAN (left column) and HSPN (right column). Typical light microscopical changes in IgAN (A,C) and HSPN (B,D) with crescents (C,D), mesangial hypercellularity and matrix expansion (A,B) were shown on PAS-stained sections. Characteristic immunohistological findings in IgAN (E,G) and HSPN (F,H) with granular mesangial staining for IgA (E,F) and C3c (G,H) showing no difference between IgAN and HSPN. Scale bar represents 100 µm.

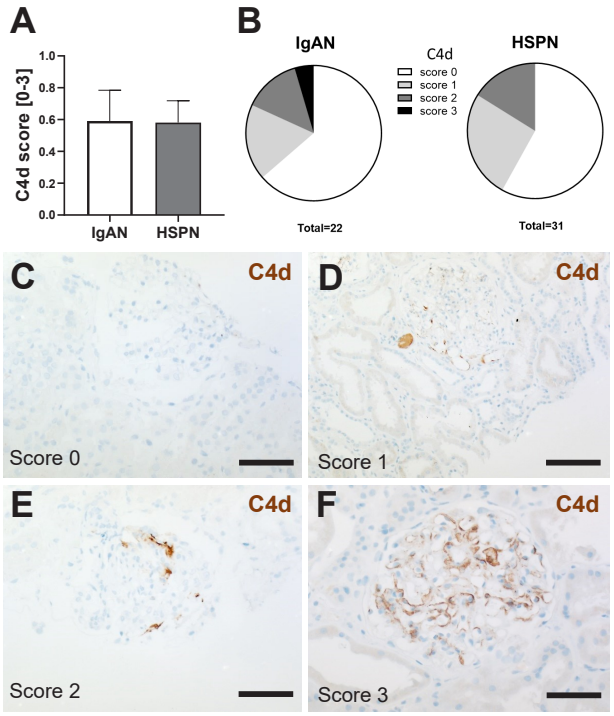

Figure 2. Evaluation of glomerular C4d-deposition in renal biopsies of pediatric patients with IgAN and HSPN. Glomerular C4d was scored semi-quantitatively (score 0-3) in renal biopsies of pediatric IgAN and HSPN patients (mean +- SD, A). Distribution of C4d scores in the IgAN and HSPN cohortes (B). Examples for C4d-negative (C) and C4d-positive (D-F, brown staining) glomerular staining allocated to score 1 (D), score 2 (E) and score 3 (F). Scale bars represents 50 µm.

C4d-negative patients, i.e. proteinuria (76%), recurrent or persistent gross hematuria (29%), and renal insufficiency (10%) in C4d-positive versus gross hematuria (50%), proteinuria (56%) and renal insufficiency (13%) in C4d-negative patients (P=N.S.).

On renal histology, there was a tendency towards higher rate of global glomerulosclerosis (29% vs. 13%, P=N.S.) and crescent formation (57% versus 38%, P=N.S.) in C4d-positive versus C4d-negative patients. The rates of segmental glomerulosclerosis and endocapillary hypercellularity were comparable between both groups. Oxford classification showed no differences between C4d-positive and C4d-negative patients. In the further follow-up, 75% of C4d-positive and 48% of C4d-negative patients received at least one immunosuppressive drug (P=N.S.). Whereas cyclophosphamide treatment was significantly more frequent in C4d-positive than C4d-negative patients (35% versus 10%, $P<0.05$), use of ACE-i was comparable (95% versus 81%, P=N.S.). At last follow-up, which was at a median of 47 months (C4d-positive patients) and 41 months (C4d-negative patients), the rate of proteinuria was similar in both groups (13% versus 15%, P=N.S.) but more C4d-positive (31%) than C4d-negative patients (12%) presented with reduced GFR <90 mL/min/1.73 m^2. Due to the small sample size this difference, however, was not statistically significant.

Discussion

The aim of our study was to compare renal biopsies of pediatric patients with HSPN and IgAN with respect to glomerular C4d-deposition and other morphological findings. As C4d is a routinely used antibody in renal transplant pathology that additionally seems to have prognostic impact in some immunecomplex GN, we were interested in whether glomerular C4d-deposition may differ between pediatric HSPN and IgAN. For IgAN, C4d-positivity was described in a variable percentage (21%-68%) of patients (4-7). Of interest, for HSPN this information is lacking as only very few studies included HSPN patients. Espinosa et al analyzed a total number of 8 HSPN patients of which 2 were positive for C4d (25%) resulting in a similar rate of C4d-positivity as in IgAN patients (32%) (4). All other studies analyzing glomerular

Table 2. Comparison of C4d-positive and C4d-negative patients

	C4d-pos (n=21)	C4d-neg (n=32)	*P* value
Age [years]	12.5 (4-18)	9.88 (2.3-16.5)	ns
Gender [male/female]	9/12	20/12	ns
History of gross hematuria [% of patients]	38.1%	67.7%	<0.05
Hypertension [% of patients]	42.9%	18.8%	ns
Laboratory findings			
Creatinine [mg/dL]	0.50 (0.29-11.27)	0.52 (0.23-3.48)	ns
GFR [mL/min/1.73m^2]	126.2 (5.7-170.5)	118.7 (21.5-176.8)	ns
Serum protein [g/L]	63 (54-83)	66 (48-80)	ns
Serum albumin [g/L]	34 (19-48)	37.8 (23-48)	ns
Serum C3 [g/L]	1.2 (0.7-1.5)	1.3 (1.0-1.7)	ns
Protein in spot urine [mg/g creatinine]	1973 (107-23565)	1000 (127-7590)	ns
Protein in 24-h urine [mg/m^2/d]	1082 (49-5980)	695 (57-3390)	ns
Renal biopsy results			
Glomerulosclerosis [% of patients]	28.6%	12.5%	ns
Segmental sclerosis [% of patients]	61.9%	56.3%	ns
Endocapillary hypercellularity [% of patients]	42.9%	34.4%	ns
Crescents [% of patients]	57.1%	37.5%	ns
Diagnosis of IgAN/HSPN	8/13	14/18	ns
Last follow up			
Time span biopsy to last follow up [months]	47 (8-115)	41 (6-118)	ns
Last follow up: GFR <90 mL/min/1.73 m^2 [% of patients]	31.3% (5/16)	11.5% (3/26)	ns
Last follow up: presence of proteinuria [% of patients]	13% (2/15)	15.4% (4/26)	ns
Treatment			
Immunosuppressive treatment [% of patients]	75%	48.4%	ns
Cyclophosphamide treatment [% of patients]	35%	9.7%	<0.05
ACE-inhibitor treatment [% of patients]	95%	80.6%	ns

GFR, glomerular filtration rate; Data shown as median (range), ns: $P > 0.05$.

C4d-deposition so far excluded HSPN patients. Therefore, our analysis is the first to analyse glomerular C4d-positivity in pediatric HSPN patients and also the largest study population for HSPN patients with respect to glomerular C4d-staining so far. Interestingly, we found a comparable rate of glomerular C4d-positivity in pediatric HSPN (42%) and IgAN patients (36%) indicating that C4d-deposition is not helpful in distinguishing between renal manifestation of HSPN and IgAN. In addition, we analyzed the differences between our HSPN and IgAN patients with regard to other histological and some clinical parameters. We found a higher rate of gross hematuria in IgAN compared to HSPN patients. In contrast, HSPN patients showed a higher rate and significant higher level of proteinuria and significantly more endocapillary hypercellularity. Recently, the CureGN study, a large register study, investigated a subgroup of 285 pediatric IgAN and HSPN patients (1). Consistent with our results, they found a higher age in pediatric IgAN compared to HSPN patients with the mean ages of both groups being very similar to our monocentric patient cohort.

The second question of our study was to compare C4d-positive versus C4d-negative patients. Here, we found a significantly lower rate of gross hematuria and significantly more cyclophosphamide treated patients in the C4d-positive compared to the C4d-negative group. In addition, there was a tendency towards more proteinuria, hypertension and immunosuppressive treatment in C4d-positive patients in general as well as C4d-positive patients slightly more often suffered from lower grade of renal insufficiency at last follow-up, i.e. 4 years after renal biopsy. Several studies in IgAN (5,7,13) showed that glomerular C4d-positivity was associated with poor renal outcome. This might be of practical relevance as staining for C4d is a standard method in renal pathology and thus widely used (3,14-16). C4d, a degradation product of complement cascade, is generated by activation of classical and lectin pathways (17,18) and present in glomerular diseases (17). Activation of the complement system plays a pathophysiological role in IgAN (18-20). Apart from activation of the alternative pathway (as evidenced by C3c deposition) (21) about 25% of IgAN patients showed glomerular deposition of mannose binding lectin (MBL) and C4d pointing to additional activation of the lectin pathway. Of note, C4d-deposits were not found to be in coincident with MBL positivity, but the subgroup of C4d-positive IgAN patients presented more severe renal damage in histology and pronounced proteinuria (21). In general, renal C4d-staining is predominantly seen in the mesangium (43%), but was also reported in tubules (11%)

and interlobular arteries (22%); in contrast to ABMR, no C4d-positive peritubular capillaries were reported in IgAN (6). In 2009, Espinosa et al were the first to systematically analyse mesangial C4d-staining in patients with histological proven IgAN and found an association of positive C4d-staining with older age, hypertension, lower gross hematuria, more glomerulosclerosis, interstitial fibrosis, lower GFR and higher proteinuria, respectively (4). When analysing long-term renal outcome they found a significantly higher 10 years renal survival in C4d-negative (91%) versus C4d-positive (44%) IgAN patients (4). Only a small number of C4d-negative patients (7.5%) developed ESRD in the mean follow-up time of 12.7 years. These results of prognostic impact of C4d positivity were confirmed in a large multicentre Spanish study of 283 patients identifying glomerular C4d-positivity as an independent risk factor for development of ESRD in IgAN (5). Additionally, in pediatric IgAN patients with less chronic renal changes and a lower rate of hypertension, 21 % showed positive glomerular C4d-staining which was an independent predictor of loss of renal function with renal survival of 8.6 years in C4d-positive versus 15.1 years in C4d-negative patients (7). In this analysis (7), C4d-positive patients showed higher levels of proteinuria, a tendency that was also seen in our study. Even in our follow-up analysis, we found more patients with at least mild renal insufficiency (GFR <90 mL/min/1.73 m^2) in the C4d-positive group (31% versus 12%, P= N.S.). Interestingly, the rate of immunosuppressive treatment was somewhat higher in C4d-positive compared to C4d-negative patients (75% versus 48%, P= N.S.) and this difference even reached statistical significance when analysing the use of cyclophosphamide separately. Both results might indicate a more severe course of HSPN and IgAN when glomerular C4d is present and are in line with the report of Segarra et al (13) who investigated C4d in a subgroup of IgAN patients with GFR >80 mL/min and found a lower amount of only 20% C4d-positivity compared to other studies (4-6). However, in this study (13) C4d-positivity was associated with higher overall proteinuria as well as in the follow up analyses more flares, more immunosuppressive treatment and higher proteinuria compared to C4d-negative patients (13). In a multivariate analysis, C4d was found to be an independent predictor of a decline in GFR slope and of ESRD (12). A recent study (22) investigated renal microangiopathic lesions in adult and pediatric IgAN and HSPN patients using vascular C4d and C5b-9 positivity as markers of complement activation. Of note, they found renal microangiopathy associated with higher vascular C4d and C5b-9 deposits. Interestingly, patients with microangiopathy and vascular C4d-positivity had a significantly worse outcome with poorer renal survival, lower GFR and increased chronic lesions on histology (22). The main analysis of this study, however, did not distinguish between pediatric and adult patients nor IgAN and HSPN patients. Of further note, in our analysis, we did not see cases of HSPN or IgAN with thrombotic microangiopathy.

The results of our monocentric retrospective study are limited by the relatively low number of pediatric patients and some dropout in the follow up which in some aspects may explain the lack of statistical significance in the existing differences between C4d-positive and C4d-negative patients. On the other hand, our single centre patients represent a relatively homogenous group in terms of diagnosis and treatment. We also have to acknowledge that C4d-staining might vary as already shown for IgAN patients (23) and therefore one has to be careful with respect to the conclusions drawn from these data. However, we tried to take care of this problem by staining all biopsies at one time point and by multiple separate analyses of the staining.

Conclusion

In conclusion, in our mono-centric study in pediatric patients glomerular C4d was not useful to differentiate between HSPN and IgAN, but was associated with a tendency to a more severe course of the disease that needs to be confirmed in larger multicentric studies.

Limitations of the study

The main limitations of our mono-centric study are the relatively low-number of pediatric patients per group and some dropout in the follow up which may explain at least in part the lack of statistical significance in some of the existing differences between C4d-positive and C4d-negative patients. We therefore suggested to analyze glomerular C4d staining in pediatric HSPN in larger multi-centric studies.

Acknowledgements

The authors thank M. Klewer, K. Schmitt, S. Söllner, A. Kosel and M. Reutelshöfer for excellent technical assistance.

Authors' contribution

Each listed author has participated sufficiently in the work to take public responsibility for the content. In conception or design, or analysis and interpretation of data, or both: KB, KA, CD, MB, KS, MG and FF. In drafting the article or revising it: KB, KA, CD and MB. In providing intellectual content of critical importance to the work described: FF and EV. Final approval of the version to be published was performed by all authors.

Conflicts of interest

The authors declare that they have no conflicts of interests.

Ethical considerations

Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

Funding/Support

The study was supported by the German Research Association (DFG, project number 387509280, SFB1350, C2) and the Deutsche Hochdruckliga (to K.B.).

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