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

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Abstract

Background

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. Aim of this study was to investigate the significances of glomerular C4d-deposition as discriminating factor between pediatric HSPN and IgAN.

Methods

We retrospectively analysed 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 were older, presented more often with gross hematuria, lower rates of proteinuria and less endocapillary hypercellularity on histology 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 lesser gross hematuria, but slightly more proteinuria, hypertension, higher rates of immunosuppressive treatment and renal insufficiency at last follow-up compared to C4d-negative patients.

Conclusions

In conclusion, glomerular C4d does not differ between pediatric HSPN and IgAN as the rate of C4d-positivity was comparable in our study. However, in individual cases of pediatric IgAN and most importantly HSPN glomerular C4d-positivity may indicate a more severe course of the disease and might thus be helpful for therapeutic decisions.

Background:

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 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 involvement, 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). 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 [3,4,5]. 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 [3]. Furthermore, glomerular C4d-positivity was shown to be an independent risk factor for development of end stage renal disease (ESRD) in adult IgAN patients [4]. In pediatric IgAN, C4d-positivity was detected in 21% of cases and was also identified as an independent predictor of worse renal outcome [6].

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 [3] 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. Therefore, it was the aim of our study to retrospectively analyse renal biopsies of pediatric patients with HSPN and IgAN from one single 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 could be used to subclassify the cases or to predict prognosis.

**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 [mmHg], 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 creatinine], urine protein in 24-hour urine [mg/m²/d]. The minimal required follow-up time after renal biopsy was 6 months. Accordingly, 11 of the 53 patients had no adequate follow-up examination as a consequence of transfer to another hospital.

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 [7,8,9]. In addition, patient records were reviewed for immunosuppressive (steroids, cyclophosphamide and others) and ACE-inhibitor (ACE-i) treatment. 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 (re.-no.4415).

- Definitions: Nephrotic-range proteinuria was defined as urinary total protein >2.0 g/g creatinine in spot urine or >1.0 g/m²/day in 24-h 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/m²/day in 24-h urine, respectively. The glomerular filtration rate (GFR) was calculated using the Schwartz formula: GFR=height [cm]×0.413/creatinine[mg/dl] [10]. Impaired renal function was defined as a GFR <90 ml/min/1.73 m². Hypertension was defined as systolic blood pressure >95th percentile [11].

- Immunostaining: We used 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). 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.

- 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. URL https://www.R-project.org/].

Results:

Patient characteristics (Table 1 + 2)
Table 1
Comparison of pediatric patients with IgAN and HSPN [data shown as median (range), ns: p > 0.05]

|                                | IgAN (n = 22) | HSPN (n = 31) | p-value |
|--------------------------------|---------------|---------------|---------|
| age [years]                    | 13.25 (6-17.5)| 9.75 (2.25-18)| p = 0.054 |
| gender [male/female]           | 14/8          | 15/16         | ns      |
| history of gross hematuria [% of patients] | 72.7%        | 43.3%         | p < 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) | p < 0.05 |
| glomerular filtration rate (GFR) [ml/min/1.73 m²] | 110.8 (5.72–176.8) | 124.8 (33.0–170.5) | p < 0.05 |
| serum protein [g/l]            | 68 (54–80)    | 63 (48–83)    | p < 0.05 |
| serum albumin [g/l]            | 41 (29–48)    | 34 (19–44)    | p < 0.005 |
| serum C3 [g/l]                 | 1.17 (0.72–1.34) | 1.28 (1.01–1.7) | p < 0.05 |
| protein in spot urine [mg/g creatinine] | 891 (107–7590) | 2157 (129–23595) | p < 0.05 |
| protein in 24 h urine [mg/m²/d] | 467.5 (49-3700) | 1140 (57-5980) | p < 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%         | p < 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 m² [% 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      |
We analysed 53 Caucasian patients, 22 (14 male, 8 female) with IgAN, 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 (Table 1, Fig. 1)

- **IgAN was associated with a higher incidence of gross hematuria, 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 vs 9.91 ± 4.11 years, n.s.) and suffered significantly more often from gross hematuria (72.7% vs 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, indication for renal biopsy in HSPN patients was proteinuria (81%) in the majority of cases and less frequently recurrent or persistent gross hematuria (26%) or renal insufficiency (3%). 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 (n.s.). Serum creatinine levels were significantly higher in the IgAN compared to the HSPN group 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 m² and 120.4 ± 33.0 ml/min/1.73 m²; 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 higher in HSPN compared to IgAN patients (Table 1). Of interest, in HSPN most patients (58%) showed nephrotic range proteinuria and only 13% had no proteinuria. In contrast, in IgAN only 23% presented with nephrotic range proteinuria and 36% had no proteinuria at the time of renal biopsy (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 g/l ± 0.17 g/l vs 1.15 g/l ± 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 vs 36% in IgAN, n.s., Fig. 1A-D). Both groups showed typical glomerular staining for IgA (Fig. 1E,F) and C3c (Fig. 1G,H). Of particular note, the percentage of endocapillary hypercellularity (Fig. 1A-D) was significantly higher in HSPN.
compared to the IgAN (52% vs 18%; p < 0.05). Positive glomerular C4d-staining was found in a similar percentage in both groups, i.e. in 36% of IgAN and 42% of HSPN (n.s.). In parallel, the more detailed evaluation of glomerular C4d-staining using a semiquantitative scoring system (0–3) showed neither differences in mean values of IgAN and HSPN (Fig. 2A), nor in distribution of score values (Fig. 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. Of note, 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 (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 m². Interestingly, a slightly higher number of HSPN patients (19%) showed proteinuria compared to IgAN (7%) (n.s.).

Laboratory findings, renal biopsy results and treatment in C4d-positive versus C4d-negative IgAN and HSPN patients (Table 2)
### Table 2
Comparison of C4d-positive and C4d-negative patients [data shown as median (range), ns: p > 0.05]

|                         | 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% | **p < 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      |
| glomerular filtration rate (GFR) [ml/min/1.73 m²] | 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/m²/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 m² [% 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:**           |                  |                  |         |
### Table 2

| Treatment                              | C4d-pos (n = 21) | C4d-neg (n = 32) | p-value |
|----------------------------------------|------------------|------------------|---------|
| Immunosuppressive treatment [% of patients] | 75%              | 48.4%            | ns      |
| Cyclophosphamide treatment [% of patients] | 35%              | 9.7%             | p < 0.05 |
| ACE-inhibitor treatment [% of patients] | 95%              | 80.6%            | ns      |

• **C4d-positivity was associated with lower incidence of gross hematuria and slightly higher extent of proteinuria, hypertension, immunosuppressive treatment and renal insufficiency at last follow-up**

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). Of note, gross hematuria was significantly more frequent in C4d-negative patients (68% vs 38%, p < 0.05). There was a tendency for higher rates of hypertension (43% vs. 19%, n.s.) and proteinuria 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 differ between the groups. Indication for renal biopsy slightly varied between C4d-positive and C4d-negative patients, i.e. proteinuria (76%), recurrent or persistent gross hematuria (29%), and renal insufficiency (10%) in C4d-positive vs gross hematuria (50%), proteinuria (56%) and renal insufficiency (13%) in C4d-negative patients (n.s.).

On renal histology, we found a slightly higher rate of global glomerulosclerosis in C4d-positive vs. C4d-negative patients (29% vs 13%, n.s.) and a tendency to a higher crescent formation (57% vs 38%, n.s.). The rate of segmental glomerulosclerosis or endocapillary hypercellularity was 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. Of interest, cyclophosphamide treatment was significantly more frequent in C4d-positive than C4d-negative patients (35% vs 10%, p < 0.05) whereas use of ACE-i was comparable (95% vs 81%, n.s.). At last follow-up, which was at a median of 47 months (C4d-positive patients) and 41 months (C4d-negative patients) more C4d-positive (31%) than C4d-negative patients (12%) presented with reduced GFR < 90 ml/min/1.73 m². Due to the small sample size this difference, however, was not statistically significant. Of note, the rate of proteinuria at follow up was similar in both groups (13% vs 15%, n.s.).

**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 some prognostic impact in some immune complex glomerulonephritis (GN), we were interested in whether mesangial C4d-deposition may differ between pediatric HSPN and IgAN. For IgAN, C4d-positivity was described in a variable percentage
(21–68%) of patients [3–6]. Of interest, for HSPN this information is lacking as only very few studies included HSPN patients. Espinosa et al. analysed 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%) [3]. All other studies analyzing glomerular C4d-deposition so far excluded HSPN patients. Therefore, our analysis is the first to analyse glomerular C4d-positivity in pediatric HSP 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. Of note, 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 higher rate of gross hematuria in C4d-positive patients as well as slightly more proteinuria, hypertension and immunosuppressive treatment. C4d-positive patients more often suffered from lower grade of renal insufficiency at last follow-up, i.e. 4 years after renal biopsy. As in other studies in IgAN [4, 6, 12] glomerular C4d-positivity was also 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 [13, 14, 15]. C4d, a degradation product of complement cascade, is generated by activation of classical and lectin pathways [16, 17] and present in glomerular diseases [16]. Activation of the complement system plays a pathophysiological role in IgAN [17, 18, 19]. Apart from activation of the alternative pathway (as evidenced by C3c deposition) [20] 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, the C4d-deposits were not found to be in coincidence with the MBL positivity, but the subgroup of C4d-positive IgAN patients presented more severe renal damage in histology and pronounced proteinuria [20]. 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 [5]. 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 [3]. When analysing long-term renal outcome they found a significantly higher 10 years renal survival in C4d-negative (91%) vs C4d-positive (44%) IgAN patients [3]. Only a small number of C4d-negative patients (7.5%) developed ESRD in the mean follow-up time of 12.7 years. These results 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 [4]. Also 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 vs. 15.1 years in C4d-negative patients [6]. In this analysis, C4d-positive patients showed higher levels of proteinuria, a tendency that was also seen in our study. Also in our follow-up analyses we found more patients with at least mild renal insufficiency (GFR < 90 ml/min/1.73 m²) in the C4d-positive group (31% vs 12%). Interestingly, the rate of immunosuppressive treatment was slightly higher in C4d-positive compared to C4d-negative patients and this difference even reached statistical significance when analysing the use of cyclophosphamide separately. Both results 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 [12] 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 [3–5]. However, C4d-positivity was associated with higher overall proteinuria. In the follow-up C4d-positive patients suffered from more flares, received more immunosuppressive treatment and had higher proteinuria compared to C4d-negative patients. 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 [21] 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 [21]. The main analysis of this study, however, did not distinguish between pediatric and adult patients nor IgAN and HSPN patients

The results of our monocentric retrospective study are limited by the relatively low number of pediatric patients and some dropout in the follow up. 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 be variable as shown for IgAN patients and therefore one has to be careful with respect to the conclusions drawn from these data. We tried to take care of this problem, however, by staining all biopsies at one time point and by multiple separate analyses of the staining.

Conclusions:

In conclusion, glomerular C4d-staining does not differ between HSPN and IgAN as the rate of C4d-positivity was comparable in both groups. The findings in our single centre pediatric HSPN patients, however, are in line with earlier studies in IgAN showing glomerular C4d-positivity to be an adverse histological marker associated with poor renal outcome.

Abbreviations

ABMR
active antibody mediated rejection
C4d
Complement factor 4d
ESRD
end stage renal disease
GFR
glomerular filtration rate
GN
glomerulonephritis
HSPN
Henoch-Schönlein purpura nephritis
IgAN
IgA nephropathy

Declarations

Ethics approval and consent to participate

The study was conducted according to the general guidelines for studies using human material. The retrospective use of archival renal biopsy material was approved by the ethics committee of the Friedrich-Alexander University (FAU) Erlangen-Nürnberg (re.-no. 4415) waving the need for informed consent.

Consent for publication

Not applicable.

Availability of data and materials

The data underlying this article will be shared on reasonable request to the corresponding author.

Competing interests

The authors declare that they have no competing interests.

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Authors’ contributions

Each listed author have 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, FF.
drafting the article or revising it: KB, KA, CD, MB. In providing intellectual content of critical importance to
the work described: FF, EV. Final approval of the version to be published was performed by all authors.

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References

[1] Selewski DT, Ambruzs JM, Appel GB et al. Clinical Characteristics and Treatment Patterns of Children
and Adults With IgA Nephropathy or IgA Vasculitis: Findings From the CureGN Study. Kidney Int Rep.
2018; 3(6):1373-1384.

[2] Jennette JC, Falk RJ, Bacon PA et al. 2012 revised International Chapel Hill Consensus Conference
Nomenclature of Vasculitides. Arthritis Rheum. 2013; 65(1):1-11.

[3] Espinosa M, Ortega R, Gómez-Carrasco JM, López-Rubio F, López-Andreu M, López-Oliva MO, Aljama P.
Mesangial C4d deposition: a new prognostic factor in IgA nephropathy. Nephrol Dial Transplant. 2009;
24(3):886-91.

[4] Espinosa M, Ortega R, Sánchez M et al. Association of C4d deposition with clinical outcomes in IgA
nephropathy. Clin J Am Soc Nephrol. 2014; 9(5):897-904.

[5] Heybeli C, Unlu M, Yildiz S, Çavdar C, Sarioglu S, Camsari T. IgA nephropathy: association of C4d with
clinical and histopathological findings and possible role of IgM. Ren Fail. 2015; 37(9):1464-9.

[6] Fabiano RCG, de Almeida Araújo S, Bambirra EA, Oliveira EA, Simões E Silva AC, Pinheiro SVB.
Mesangial C4d deposition may predict progression of kidney disease in pediatric patients with IgA
nephropathy. Pediatr Nephrol. 2017; 32(7):1211-1220.

[7] Coppo R, Troyanov S, Camilla R et al. The Oxford IgA nephropathy clinicopathological classification is
valid for children as well as adults. Kidney Int 2010; 77: 921–927.

[8] Coppo R, Troyanov S, Bellur S et al. Validation of the Oxford classification of IgA nephropathy in
cohorts with different presentations and treatments. Kidney Int. 2014; 86(4):828-36.

[9] Cattran DC, Coppo R, Cook HT et al. The Oxford classification of IgA nephropathy: rationale,
clinicopathological correlations, and classification. Kidney Int. 2009; 76(5):534-45.

[10] Schwartz GJ, Work DF. Measurement and estimation of GFR in children and adolescents. Clin J Am
Soc Nephrol 2009; 4: 1832–1843.
[11] Neuhauser HK, Thamm M, Ellert U, Hense HW, Rosario AS. Blood pressure percentiles by age and height from nonoverweight children and adolescents in Germany. Pediatrics. 2011; 127(4): e978-88.

[12] Segarra A, Romero K, Agraz I et al. Mesangial C4d Deposits in Early IgA Nephropathy. Clin J Am Soc Nephrol. 2018; 13(2):258-264.

[13] Collins AB, Schneeberger EE, Pascual MA et al. Complement activation in acute humoral renal allograft rejection: diagnostic significance of C4d deposits in peritubular capillaries. J Am Soc Nephrol. 1999; 10(10):2208-14.

[14] Sapir-Pichhadze R, Curran SP, John R et al. A systematic review of the role of C4d in the diagnosis of acute antibody-mediated rejection. Kidney Int. 2015; 87(1):182-94.

[15] Solez K, Colvin RB, Racusen LC et al. Banff '05 Meeting Report: differential diagnosis of chronic allograft injury and elimination of chronic allograft nephropathy ('CAN'). Am J Transplant. 2007; 7(3):518-26.

[16] Chandra P. C4d in native glomerular diseases. Am J Nephrol 2019; 49:81-92.

[17] Daha MR, van Kooten C. Role of complement in IgA nephropathy. J Nephrol. 2016; 29(1):1-4.

[18] Maillard N, Wyatt RJ, Julian BA, Kiryluk K, Gharavi A, Fremeaux-Bacchi V, Novak J. Current understanding of the role of complement in IgA nephropathy. J Am Soc Nephrol. 2015; 26(7):1503-12.

[19] Otani M, Nakata J,Kihara M, Leroy V,Moll S,Wada Y, Izui S. O-glycosylated IgA rheumatoid factor induces IgA deposits and glomerulonephritis. J Am Soc Nephrol 2012; 23: 438–446.

[20] Roos A, Rastaldi MP, Calvaresi N et al. Glomerular activation of the lectin pathway of complement in IgA nephropathy is associated with more severe renal disease. J Am Soc Nephrol 2006; 17: 1724–1734.

[21] Chua JS, Zandbergen M, Wolterbeek R et al. Complement-mediated microangiopathy in IgA nephropathy and IgA vasculitis with nephritis. Mod Pathol. 2019; 32(8):1147-1157.

**Figures**
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) as well as 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 100µm.

**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 cohorts (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 represent 50 µm.