Outcome of immunosuppression in children with IgA vasculitis-related nephritis

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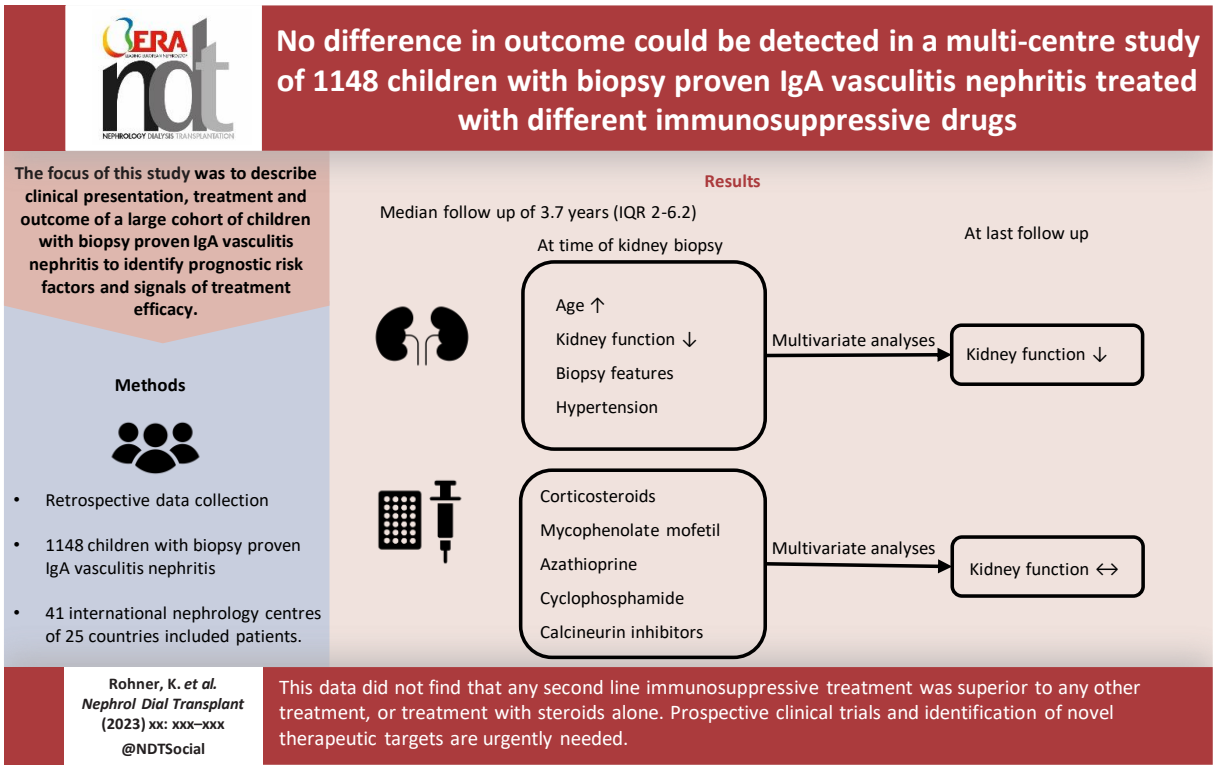
Running Head

Outcome of IgA vasculitis nephritis in children

Graphical Abstract



Abstract

Background and hypothesis: IgA vasculitis with nephritis (IgAVN) is the most common vasculitis in children. Treatment recommendations are, due to a lack of evidence, based on expert opinion resulting in variation. The aim of this study was to describe clinical presentation, treatment and outcome of an extremely large cohort of children with biopsy proven IgAVN to identify prognostic risk factors and signals of treatment efficacy.

Methods: Retrospective data were collected on 1148 children with biopsy proven IgAVN between 2005 and 2019 from 41 international paediatric nephrology centres across 25 countries and analyzed using multivariate analysis. The primary outcome was estimated glomerular filtration rate (eGFR) and persistent proteinuria at last follow up.

Results: The median follow up was 3.7 years (IQR 2-6.2). At last follow up, 29% of patients had an eGFR <90ml/min/1.73m^2, 36% had proteinuria and 3% had chronic kidney disease stage 4-5. Older age, lower eGFR at onset, hypertension and histological features of tubular atrophy and segmental sclerosis were predictors of poor outcome. There was no evidence to support any specific second line immunosuppressive regimen to be superior to others, even when further analysing subgroups of children with reduced kidney function, nephrotic syndrome or hypoalbuminemia at onset. Delayed start of immunosuppressive treatment was associated with a lower eGFR at last follow up.

Conclusion: In this large retrospective cohort, key features associated with disease outcome are highlighted. Importantly there was no evidence to support that any specific immunosuppressive treatments were superior to others. Further discovery science and well-conducted clinical trials are needed to define accurate treatment and improve outcomes of IgAVN.

Key learning points:

What was known:

- IgA vasculitis nephritis (IgAVN) in children is self-limiting in the majority of cases but a proportion progress to chronic kidney disease.
- Evidence base guiding treatment decisions is limited.

This study adds:

- This study compares outcomes in children treated with corticosteroids, mycophenolate mofetil, calcineurin inhibitor, cyclophosphamide, azathioprine, rituximab and plasmapheresis in a large population of children.
- No immunosuppressive treatment was found to be superior to any of the others.

Potential impact:

- Optimal treatment strategy of IgAVN in children remains unclear
- Further discovery science and well-conducted clinical trials are needed.

Keywords

IgA vasculitis nephritis, children, immunosuppression, Henoch-Schonlein purpura nephritis

Abbreviations

ACE	Angiotensin converting enzyme
CKD	Chronic kidney disease
CNI	Calcineurin inhibitor
eGFR	estimated glomerular filtration rate
IgAV	IgA vasculitis
IgAVN	IgA vasculitis nephritis
IQR	Interquartile range
KDIGO	Kidney Disease Improving Global Outcome
KRT	Kidney replacement therapy
MMF	Mycophenolate mofetil
NS	Nephrotic syndrome
RAS	Renin-Angiotensin system
RCT	Randomized controlled trial
SHARE	Single Hub and Access point for paediatric Rheumatology in Europe
UPUC	urinary protein/creatinine ratio

Introduction

IgA vasculitis (IgAV), previously known as Henoch-Schönlein Purpura is the most common vasculitis in childhood[1] and the EULAR/PRINTO/PRES criteria are used to classify IgAV[2]. Nephritis is ~~a common manifestation of IgAV. It is~~ reported in about one third of children with IgAV[3]. Similar to the other IgAV manifestations, nephritis is self-limiting in the large majority of children[4]. A proportion of children, however, develop chronic haematuria and proteinuria and a small number develop worsening kidney function and even kidney failure[5].

Clinical factors, mainly the initial kidney function and degree of proteinuria, and histological findings suggestive of chronicity on the kidney biopsy, have been shown to potentially predict outcome.[4] Treatment of IgAV nephritis (IgAVN) is controversial. Due to a lack of evidence, treatment recommendations are based on a few randomised trials and several case series. Kidney Disease Improving Global Outcome (KDIGO) recommends that children with persistent proteinuria after 3 months are treated with angiotensin converting enzyme (ACE) inhibition or an angiotensin receptor blocker. KDIGO recommends further treatment with oral prednisolone or intravenous methylprednisolone for children with mild or moderate IgAVN and suggests that children with nephrotic syndrome or rapidly deteriorating kidney function should receive additional immunosuppressive agents like cyclophosphamide, mycophenolate mofetil (MMF) or rituximab similar to the management of ANCA-associated vasculitis[6].

Cyclophosphamide, ciclosporin, tacrolimus, MMF and rituximab have all been reported to successfully improve kidney outcome of IgAVN[7-10]. The SHARE (Single Hub and Access point for paediatric Rheumatology in Europe) initiative on IgAV produced 19 different

recommendations for the treatment of this condition with all recommendations, apart from one, being based on expert agreement[11].

The primary aim of this study was to describe clinical presentation, treatment and outcome in a large cohort of children with biopsy proven IgAVN to identify any potential prognostic factors and/or signals of treatment efficacy.

Material and Methods

Participants and data collection

Children younger than 18 years at time of kidney biopsy, diagnosed between 2005 and 2019, with typical clinical symptoms of IgAV meeting the EULAR/PRINT/PRES criteria,[2] at least one histological classification of the kidney biopsy and a follow up of at least 12 months after initial presentation were included. The decision to perform a kidney biopsy had been made ~~by each nephrologist~~ using local criteria.

Data were collected between December 2020 to August 2021. Electronic invitations to contribute were sent to members of the European Society for Paediatric Nephrology and the International Paediatric Nephrology Association. All data were fully anonymised to comply with international regulations including General Data Protection Regulation. Each individual centre was responsible for applying local ethical requirements to collect retrospective data. Data were entered by local investigators into standardised ~~study~~data sheets. Completed data sheets were sent via email or secure platforms and data transfer agreements were organised as required.

Clinicians were asked to enter all patients biopsied in their centre during the study period, to avoid potential selection bias. Registered data included baseline demographic data, biopsy classification International Study of Kidney Diseases in Children (ISKDC)[12] and/or Oxford Classification (MEST-C score)[13], treatment received ~~including time point~~ and ~~follow-up~~ data at ~~6, 12, 24 months and at~~ last available follow-up ~~(2 months before and after the timepoint were accepted)~~. The primary outcome was estimated glomerular filtration rate (eGFR) and proteinuria at last follow-up.

Chronic kidney disease (CKD) was staged according KDIGO[14].

Definitions and data handling

Data were collated centrally and patients not fulfilling the inclusion criteria were excluded from the analyses.

The modified Schwartz Formula was used to calculate eGFR[15]. For patients missing a height the 50th percentile of the WHO child growth standards for the according age and gender was taken to calculate GFR[16]. GFR of patients with kidney transplantation was assumed to be 5ml/min/1.73m^2 at the follow up ~~time points~~. Arterial hypertension was defined by values above the 95th centile for age and height according to the 2016 European Society of Hypertension guidelines[17].

Proteinuria was defined as protein/creatinine ratio (UPUC) >20mg/mmol (177mg/g) and nephrotic range proteinuria as UPUC >200mg/mmol (1770mg/g). Hypoalbuminemia <25g/l in combination with UPUC >200mg/mmol was considered as nephrotic syndrome (NS) even if data on clinical symptoms as oedema had not been collected[6]. Urinary albumin/creatinine ratio was transformed to UPUC with a conversion factor of 1.43. No validated conversion formula for children could be found in the literature. The factor was calculated from the children in this study with both parameters available at first presentation. 24hour-protein-measurement (g/24h) data was transformed to UPUC in mg/mmol[18].

Treatment was classified into three groups: no treatment, treatment given for 6 weeks or less, and treatment given for longer than 6 weeks. Only treatments given for more than 6 weeks were regarded as ongoing treatment for the purposes of the statistical analyses to

avoid that treatments given for a very short period of time were counted as active treatments. Rituximab was regarded as given after the first dose and plasmapheresis when more than 4 sessions had been performed.

We performed subgroup analysis of children with eGFR below 60 and 90ml/min/1.73m^2,~~respectively,~~ hypoalbuminemia (serumalbumin <35g/l) and with NS ~~as previously defined~~. Further subgroup analyses compared different second line immunosuppressive drugs with each other and steroid treatment.

Statistical analyses

Statistical analyses were performed using SPSS® Version 29 (IBM Corporation, Armonk, NY, USA). Descriptive statistics were expressed as mean and standard deviation (SD) or median and inter-quartile range (IQR) where data were not normally distributed. Categorical data are given as frequency (percentage of all patients with available data).

Chi Square Tests were performed between the different treatment groups. A p-value of <0.05 was considered as statistically significant.

Minimum data entry for inclusion at any timepoint included eGFR or proteinuria at onset, at least one biopsy score, one follow-up after 12 months and data on treatment.

Potential clinical predictors of the primary outcomes were assessed initially in univariate analyses and if suggested as clinically relevant also in multivariate analysis. Univariate analysis of individual potential clinical predictors was performed using linear regression model. Potentially relevant clinical predictors and statistically significant parameters in the univariate analysis entered into linear regression model for multivariate analysis were: age, gender, ethnicity, biopsy category (Oxford classification or ISKDC), eGFR, UPUC,

hypertension, NS, time from onset disease to start treatment and different treatments. Two separate analyses with the two different biopsy scores were performed to maximize inclusion. Multivariate analyses were also repeated for different subgroups where a potential for a positive response was regarded as higher than in the total group: impaired kidney function (eGFR <90ml/min/1.73m^2); moderately and severely impaired kidney function (eGFR <60ml/min/1.73m^2), NS and hypoalbuminemia <35g/l at onset.

The potential effects of the different immunosuppressive treatments were further analysed in subgroups. Multivariate analyses were performed between the different treatment groups (steroids alone, additional MMF, azathioprine, cyclophosphamide or calcineurin inhibitor (CNI) and use of more than 2 immunosuppressants).

To analyse potential effects of secondary immunosuppressive treatments the study population was divided into 4 subgroups depending on the severity at onset and outcome: with mild nephritis (eGFR >90ml/min/1.73m^2, ~~or~~ UPUC <200mg/mmol) versus more severe nephritis (eGFR <90ml/min/1.73m^2, ~~or~~ UPUC >200mg/mmol) and those two groups were further divided into children who did worse respectively recovered kidney function ~~or~~ respectively proteinuria (Supplementary Figure S1).

Results

This study retrospectively evaluated 1148 patients with biopsy proven IgAVN from 41 international paediatric nephrology centres in 25 countries across 5 continents (Supplementary Table S1). A total of 101 patients (101/1249; 8%) were excluded due to minimal data entry requirements. The median age, at kidney biopsy, was 8.7 years (IQR 6.3-11.7), 43.3% were female. The median duration of follow-up was 3.7 years (IQR 2-6.2). Demographic baseline data, laboratory characteristics and results of biopsy scores are summarized in Table 1 for the total group and in Supplementary Table S2 for treatment subgroups.

At the time of kidney biopsy 72.1% of patients had normal eGFR while 3.3% had an eGFR below 30ml/min/1.73m^2. Proteinuria was present in the nephrotic range in 62.7%. Hypoalbuminemia (<35g/l) was detected in 50.5% of patients and 13.3% had a serum albumin below 25g/l.

Treatment

The median time from onset of IgAV to start of treatment was 28.5 days (IQR 8-72). ACE-inhibitors were given to 80.1% of children. Intravenous steroids followed by at least 6 weeks of oral steroid treatment was given to 42.9% of the patients and 38.6% received oral steroid treatment alone. Additional drugs used included MMF (13%), azathioprine (11.9%), cyclophosphamide (17.3%), CNI (10.1%), intravenous immunoglobulins (IVIG) (0.6%), rituximab (0.9%) and anticoagulants (10.5%) (Table 1). ~~Forty four percent of the children received~~ Only one immunosuppressive agent was given to 44% of the children, while 27%, 11.6%, 3.8% and 0.9 % respectively received two, three, four or more than four

immunosuppressive drugs. Immunosuppressive therapy was not used in 157 (13%) of the children.

Outcome

At last follow-up 70.8% of the 920 patients with available data had an eGFR >90ml/min/1.73m^2, and in 121 (13.6%) eGFR was >135ml/min/1.73m^2 suggestive of hyperfiltration[19]. CKD stage 2 and 3 was found in 26%, Stage 4 and 5 or having had a kidney transplantation in 3.3%. Proteinuria was persistent in 35.6% (Table 3).

Kidney replacement therapy (KRT) was needed in 7 children (0.6%) within the first three months after biopsy and in 23 children (2%) as chronic KRT (5 haemodialysis, 12 peritoneal dialysis, 6 kidney transplantation). Median time from biopsy to start of KRT was 27 months (IQR 3.3-59). Only one of the patients needing acute dialysis remained on chronic KRT.

Predictors of renal outcome

In the univariate analysis older age, male gender, hypertension, lower eGFR and NS at onset predicted lower eGFR at last follow-up. Endocapillary hypercellularity (E1) segmental sclerosis (S1), tubular atrophy/interstitial fibrosis >25% (T1 and 2) and crescents (C1 and 2) in the Oxford classification and ISKDC stages IV and V, compared to III were also associated with worse eGFR. Proteinuria remained higher in patients with older age and lower eGFR at onset and so did positive Oxford Classification S, T and C. There was no significantly positive effect of any individual immunosuppressive treatment in the univariate analysis (Table 3, Supplementary Table S3).

Multivariate analyses

In the multivariate analyses of the children whose biopsies were scored according to Oxford classification, older age, lower eGFR at onset, hypertension and tubular atrophy in the biopsy remained predictors for a worse outcome in eGFR, as did treatment with MMF, CNI and rituximab. None of the treatments were associated with an improved outcome regarding kidney function. Older age, Oxford classification items S and T positivity, and treatment with MMF and CNI remained significant predictors for increased proteinuria. MEST-C Score E was associated with a lower level of proteinuria (Table 4a). On multivariate analyses including the ISKDC Score the findings were similar. Age, hypertension, eGFR and UPUC at onset were associated with lower eGFR at follow-up. Only age was significantly associated with higher proteinuria (Table 4b).

Subgroup analyses severity of onset disease

We performed subgroup analyses to determine whether there was any treatment benefit in specific clinical subgroups of children; eGFR below 60, and 90ml/min/1.73m^2 respectively, NS and hypoalbuminemia ~~less than 25g/l and~~ <35g/l respectively. Baseline data for these subgroups are summarized in Supplementary Table S4. No statistically significant ~~association~~ improvement of outcome could be detected in eGFR or proteinuria between the different treatments used in any of the four groups (Table 5).

Comparison between the different immunosuppressive treatments

To study any positive effect of second line immunosuppressive drugs on outcome we compared patients treated with MMF, azathioprine, cyclophosphamide, CNI or a combination of those between each other, with patients treated with steroids only. eGFR at biopsy was also included in the multivariate analyses as previous analyses had revealed this to be the strongest predictor of clinical outcome in this study.

At last follow-up the eGFR was significantly worse in patients treated with more than two immunosuppressive drugs (coefficient -19.60[-25.9;-13.3], p<0.001) and CNI (~~coefficient~~ -8.17[-16.2-0.2], p=0.046) compared to treatment with steroids alone. No difference in kidney function could be detected between children treated with MMF, azathioprine and cyclophosphamide (Figure 1A). Patients treated with multiple immunosuppressive drugs (~~coefficient~~ 49.34[30.7;68.0], p<0.001) showed significantly higher proteinuria compared to steroid treatment alone (Figure 1B).

Further sub analyses

In order to analyse the effect of second line immunosuppression with MMF, azathioprine, cyclophosphamide and CNI in more detail, patients were stratified to four groups based on eGFR and proteinuria at onset and at follow-up. In the group with normal eGFR at onset, more patients treated with MMF and CNI had abnormal eGFR at follow-up compared to patients treated with azathioprine or cyclophosphamide and more patients within the MMF group had persistent proteinuria (Table 5). Within the group with abnormal eGFR at onset (eGFR <90ml/min/1.73m^2) no difference in outcome was detected between the different treatments. Similar findings were seen when cut-off of 60ml/min/1.73m^2 was applied (Data not shown).

There was no difference in eGFR at last follow-up for patients treated with intravenous steroids compared to oral steroids only when adjusted for eGFR at onset in the multivariate analysis (~~coefficient~~ -1.25[-5.4;2.9], p=0.553). Patient treated with intravenous steroids had higher proteinuria than those treated with only oral steroids (13.10[1.0;25.3], p=0.035).

Effect of time at start of treatment on outcome

Treatment was started at a median time of 28.5 (IQR 8-72) days after onset of IgAV, Children with a later start of treatment had a lower eGFR at last follow-up in both the univariate and multivariate analysis but time from start of treatment did not affect the degree of proteinuria (Table 3 and 4). In the subgroup analysis of the different severity groups (eGFR at biopsy <90 respectively <60ml/min/1.73m^2, NS and hypoalbuminemia <35g/l~~,~~ ~~respectively~~) later start of treatment was associated with lower eGFR only in the subgroup with eGFR <90ml/min/1.73m^2 (~~Coefficient~~ -0.01[-0.0;-0.0], p=0.024, n=176) at biopsy.

Discussion

We present the largest international retrospective study of biopsy proven IgAVN in children. ~~Within a cohort of 1148 children o~~Older age at onset, hypertension, lower eGFR at onset and tubular atrophy in the biopsy were predictors for worse kidney function. A meta-analysis by Shi et al. showed similar effects of eGFR and age[20]. Contrary to other studies, we found no association between proteinuria ~~at onset~~ and kidney outcome[21, 22].

Only a small proportion of the children, 3.2%, developed severely impaired kidney function ~~defined as~~ (eGFR <30ml/min/1.73m^2). A much larger group showed evidence of chronic kidney disease. In previous cohorts kidney failure was found in 4-21% whereas 67-74% had normal eGFR with no or minor urinary findings[5, 23, 24]. The baseline characteristics and clinical outcomes in our study were thus comparable to other retrospective cohorts.

None of the immunosuppressive treatments were significantly associated with any difference in clinical outcome. These findings were found in the main analyses of our study and confirmed ~~when~~ in specific subgroups (children possibly more amenable to treatment) ~~were analysed separately~~. Neither children with mildly or moderately impaired eGFR at onset nor children with ~~serum albumin in the nephrotic range~~NS showed any significant benefit from the different treatments compared to other treatments.

These findings need to be interpreted with caution as the study was retrospective and some of the treatment sub-groups were small. Our findings are however in agreement with the evaluations of the existing literature for immunosuppressive treatment made by KDIGO, SHARE and Cochrane. The most recent Cochrane review on the treatment of IgAVN could not evaluate steroid treatment due to lack of studies[25].

There are very few RCTs on immunosuppressive treatment in IgAVN in children, some are focussing more on prevention of kidney involvement rather than on treatment[26-29]. ~~A trial by~~ Jauhola et al. compared 11 patients treated with ciclosporin with 13 with iv methylprednisolone[30]. ~~The latter group responded slower and 6 patients with IV methylprednisolone vs none of the ciclosporin group needed second line treatment.~~ They concluded that treatment with ciclosporin is safe and not inferior to iv methylprednisolone. Koskela et al. confirmed this when comparing methylprednisolone pulses and ciclosporin in ~~the treatment of~~ severe IgAVN in a follow-up study with mean follow-up time of 10.8 years[31]. Another trial compared supportive treatment alone to additional cyclophosphamide in severe IgAVN. No difference could be seen in outcome of the two groups of 28 patients each[32].

Steroid treatment was used in a majority of children in our cohort (81%). As in other studies analysis of a treatment effect was therefore not possible[33]. In the study of Wakaki et al. treatment of 25 patients with steroids could not be detected as a significant predictor for outcome compared to 17 not treated patients[34]. Methylprednisolone pulses improved kidney outcome in 38 severely affected children ~~and decreased active lesions in kidney biopsy~~ but without a direct control group[35]. In our study we could not detect a clear benefit of steroid pulses compared to oral steroids only. Data on steroid doses used was not collected in this study.

None of the second line immunosuppressive treatments in our study showed a clear benefit compared to steroid treatment alone. This result needs to be interpreted with caution. Despite that the multivariate analysis adjusted for eGFR and that different subgroup analysis

were performed we cannot rule out in a retrospective study that patients treated with two immunosuppressants could have had a more severe disease.

In a subgroup analysis less patients than expected treated with MMF and CNI had a preserved kidney function among the children with normal eGFR at onset. There was no difference in eGFR at outcome in the group with impaired kidney function at onset. This suggests that future studies need to stratify the children included based on their eGFR at onset.

Du et al. reported a benefit of MMF in a case series of 12 children with severe nephritis ~~resistant to steroids and RAS blockade~~[36] and Nikibakhsh et al. in 3 children[37] but without a control group. In adult patients a retrospective study reported a higher remission rate of MMF and low dose prednisone compared to ~~a group who got only~~ high dose steroids only[38]. We did not detect any beneficial effect of MMF treatment in our cohort but in subgroup analyses there seemed to be a trend to worse outcome in MMF treated patients compared to other second line immunosuppressive drugs.

Azathioprine in combination with steroids showed a favourable outcome in 24 of 26 patients in 2 paediatric retrospective studies[39, 40]. In our study a relatively high number (137 patients) were treated with azathioprine but no superiority to steroid treatment alone or any other second line immunosuppressive drug could be found.

Treatment with cyclophosphamide is based on a randomised trial in children and on studies in adults that could not detect any benefit of additional cyclophosphamide to high dose corticosteroid treatment ~~only~~[41, 42]. Flynn et al. reported a case series of 12 children

treated with high dose steroids and ~~additional~~ oral cyclophosphamide with a seemingly beneficial effect on proteinuria[43].

Treatment with CNI has been analysed in a few studies with severe IgAVN including the randomised trial by Jauhola et al. Data of 46 children treated with ciclosporine showed a favourable outcome with remission or partial remission in all ~~of~~ children without any control group[8, 9, 30, 44].

Early treatment after biopsy was associated with a higher eGFR at last follow-up in our cohort. This is consistent with the opinion that it is important to reduce the inflammation in the glomeruli as early as possible to avoid damage[45, 46]. It is also shown that histological changes occur early in the disease course in children with renal involvement, suggesting early treatment[47].

The main strength of our study is the inclusion of a very large number of children with biopsy proven IgAVN. We were also able to look for associations with different immunosuppressive drugs. The major weakness is the retrospective nature which lead to information and selection bias. Diagnostic and treatment decisions were not standardized and the availability of drugs is likely to be different between countries. Due to a potential reverse causation bias despite using multivariate analysis, we have chosen to interpret differences in outcome in patients treated with different drugs very cautiously.

In conclusion, the data from our very large cohort of children with biopsy proven IgAVN did not find that any second line immunosuppressive treatment was superior to any other second line treatment, or treatment with steroids alone. Prospective clinical trials and identification of novel therapeutic targets are urgently needed.

Data availability statement: Original data can be provided if requested.

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Table 1: Demographic and laboratory characteristics at time of biopsy

			Patients, n = 1148
Females			497 (43.3%)
Age at biopsy, years			8.7 (6.3-11.7)
Ethnicity			
	Caucasian		493 (42.9%)
	East Asian		250 (21.8%)
	South Asian		50 (4.4%)
	Turkish		228 (19.9%)
	Other		52 (4.5%)
	Unknown (not asked)		75 (6.5%)
Duration from onset of IgAV to biopsy, months			1 (0-3)
Systolic blood pressure >95th centile			222 (20.2%)
eGFR, ml/min/1.73 m^2			109.9 (87.8-133.4)
	>90		820 (72.1%)
	60-90		195 (17.2%)
	30-60		84 (7.4%)
	15-30		23 (2.0%)
	< 15		15 (1.3%)
Serum albumin, g/L			34.0 (29-39.6)
	Hypoalbuminemia <35		560 (50.5%)
	Hypoalbuminemia <25		147 (13.3%)
Urine proteine/creatinine ratio, mg/mmol			317.5 (122.6-627.6)
	UPUC>20		1079 (95.7%)
	UPUC > 200		708 (62.7%)
Nephrotic syndrome			139 (12.6%)
Positive urine dipstick for blood			1043 (95.6%)
Biopsy (MEST C Score)			
	M	0	210 (25 %)
		1	629 (75 %)
	E	0	430 (51%)
		1	413 (49%)
	S	0	509 (60.5%)
		1	333 (39.5%)
	T	0	770 (91.4%
		1	65 (7.7%)
		2	7 (0.8%)
	C	0	385 (48.5%)
		1	334 (42.1 %)
		2	74 (9.3%)

Biopsy grade (ISKDC)

1	41 (4.1%)
2	344 (34.4%)
3	533 (53.3%)
4	52 (5.2%)
5	17 (1.7%)
6	13 (1.3%)

Treatment

Modality/Medication	Duration/doses	n
No treatment		30 (2.6%)
ACE-inhibition	>6 weeks	919 (80.1%)
Oral steroids	>6 weeks	443 (38.6 %)
	<6 weeks	17 (1.5%)
Pulses+oral steroids	>6 weeks	493 (42.9%)
	<6 weeks	32 (2.8%)
Mycophenolate mofetil	>6 weeks	149 (13.0%)
Calcineurin inhibitor	>6 weeks	116 (10.1%)
Cyclophosphamide	>6 weeks	199 (17.3%)
Azathioprine	>6 weeks	137 (11.9%)
Immunoglobulins	>6 weeks	7 (0.6%)
	<6 weeks	11 (1.0%)
Anticoagulation	>6 weeks	121 (10.5%)
	<6 weeks	9 (0.8%)
Rituximab	1 dose	1 (0.1%)
	2 doses	2 (0.2%)
	3 doses	4 (0.3%
	>3 doses	4 (0.3%)
Plasmapheresis	<4 sessions	3 (0.3%)
	4-7 sessions	6 (0.5%)
	7 sessions	7 (0.6%)
Other	>6weeks	127 (11.1%)

IgAV IgA Vasculitis, eGFR estimated glomerular filtration rate, UPUC urinary protein/creatinine ratio, ISKDC International study of Kidney Disease in Children.

Table 2: Laboratory characteristics at last follow-up:

Time from biopsy to last follow-up (years)	3.7 (2-6.2)
Systolic blood pressure, mmHg	110 (100-119)
Hypertension	66 (6.6%)
Serum creatinine, µmol/L	54.8 (44.2-69.0)
eGFR, ml/min/1.73 m^2	101.7 (87.5-121.9)
>90	651 (70.8%)
60-90	221 (24.0%)
30-60	18 (1.9%)
15-30	5 (0.5%)
<15	25 (2.7%)
Serum albumin, g/L	43.0 (41-46)
Hypoalbuminemia <35	22 (3.0%)
Hypoalbuminemia <25	0
Positive urine dipstick for blood	310 (30.2%)
Urine protein/creatinine ratio, mg/mmol	
<20	656 (64.4%)
20-200	336 (33.0%)
>200	26 (2.6%)

eGFR estimated glomerular filtration rate, *UPUC urinary protein/creatinine ratio*

Table 3: Factors at biopsy influencing eGFR and UPUC at last follow-up. Univariate analysis. Only factors with significant influence in one of the two outcome parameters are shown. In grouped parameters (ethnicities and biopsy ISKDC classification) one parameter has been chosen by the statistical program SPSS as reference.

		Influence on eGFR		Influence on UPUC	
Influencing factor		Coefficient [CI]	p	Coefficient [CI]	P
Age		-2.86 [-3.4;-2.3]	<0.001	3.12 [1.8;4.5]	<0.001
Gender (Female as reference)		-6.61 [-10.6;-2.6]	0.001	1.97 [-8.7;12.6]	0.717
Ethnicities (Caucasian as reference)	Turkish	-1.32 [-6,4;3.7]	0.610	-19.34 [-32.0;-6.7]	0.003
Biopsy Oxford Classification	E	-6.28 [-11.0;-1.6]	0.009	-3.60 [-15.5;8.3]	0.553
	S	-7.15 [-11.9;-2.4]	0.003	25.97 [14.0;38.0]	<0.001
	T (0 vs 1 and 2)	-18.31 [-26.2;-10.4]	<0.001	38.26 [18.0;58.6]	<0.001
	C (0 vs 1 and 2)	-5.95 [-10.8;-1.1]	0.015	13.53 [1.2;25.9]	0.032
Biopsy ISKDC	II	6.19 [1.5;10.8]	0.009	-12.47 [-24.7;-0.3]	0.045
(III as reference)	IV	-13.96 [-23.1;-4.9]	0.003	9.27 [-15.8;34.3]	0.468
	V	-27.66 [-42.3;-13.0]	<0.001	26.99 [-16.4;70.4]	0.222
eGFR at biopsy		0.32 [0.3;0.4]	<0.001	-0.21 [-0.3;-0.1]	0.001
NS at onset		-5.90 [-11.8;-0.0]	0.049	1.06 [-15.0;17.1]	0.897
Hypertension		-8.58 [-13.6;-3.6]	<0.001	9.38 [-4.2;22.9]	0.174
ACE- Inhibition		-0.30 [-5.7;5.1]	0.913	15.56 [2.0;29.1]	0.024
Mycophenolate mofetil		-17.15 [-23.1;-11.2]	<0.001	58.48 [42.2;74.7]	<0.001
Cyclophosphamide		-10.00 [-15.3;-4.7]	<0.001	25.45 [11.4;39.5]	<0.001
Calcineurin inhibitor		-15.9 [-22.3;-9.4]	<0.001	44.96 [27.3;62.6]	<0.001
Rituximab		-30.78 [-49.1;-12.4]	0.001	210.14 [158.3;262.0]	<0.001
Time onset to treatment		-0.01 [-0.0;-0.0]	<0.001	0.01 [-0.0;0.0]	0.068

eGFR estimated glomerular filtration rate, UPUC urinary protein/creatinine ratio, CI Confidence interval 95%, ISKDC international study of kidney disease in children, NS nephrotic syndrome, ACE angiotensin converting enzyme

Table 4: Multivariate analysis of factors at biopsy influencing eGFR and UPUC at last follow up.
Only significant factors are shown in the tables

a) Patient included with available data on Biopsy oxford classification (MEST C score):

Influencing factor	Subgroup	Influence on eGFR (n = 519)		Influence on UPUC (n = 573)	
		Coefficient [CI]	p	Coefficient [CI]	p
Age at onset		-1.97 [-2.6;-1.4]	<0.001	2.57 [0.6;4.6]	0.012
Ethnicities	East Asian	-7.87 [-13.9;-1.8]	0.011	-5.30 [-24.3;13.7]	0.584
	Turkish	-16.04 [-22.0;-10.1]	<0.001	-13.64 [-33.4;6.1]	0.176
	Other	10.59 [1.5;19.7]	0.023	2.87 [-27.9;33.7]	0.855
Biopsy Oxford (MEST C)	E	-3.21 [-7.6;1.2]	0.151	-15.09 [-29.2;-1.0]	0.036
	S	-2.55 [-7.1;2.0]	0.273	15.70 [0.9;30.5]	0.038
	T (0 vs 1 and 2)	-9.88 [-17.2;-2.6]	0.008	27.47 [3.4;51.4]	0.025
Hypertension		-5.24 [-10.5;-0.2]	0.049	-12.12 [-29.2;5.0]	0.164
eGFR at biopsy		0.26 [0.2;0.3]	<0.001	-0.07 [-0.3;0.1]	0.507
Mycophenolate mofetil		-6.30 [-12.6;-0.0]	0.048	40.48 [19.2;61.8]	<0.001
Azathioprine		-7.11 [-13.5;-0.8]	0.029	7.72 [-13.3;28.8]	0.471
Calcineurin inhibitor		-17.37 [-25.5;-9.2]	<0.001	69.45 [42.0;96.8]	<0.001
Rituximab		-25.61 [-46.1;-5.1]	0.014	-6.18 [-82.6;70.2]	0.874
Time onset to treatment		-0.01 [-0.0;-0.0]	<0.001	0.01 [-0.0;0.0]	0.449

b) Patients included with data on ISKDC biopsy score

Influencing factor	Subgroup	eGFR at last follow up (n=644)		UPUC at last follow up (n=719)	
		Coefficient [CI]	p	Coefficient [CI]	p
Age at onset		-1.68 [-2.3;-1.1]	<0.001	1.81 [0.2;3.4]	0.030
Biopsy ISKDC (Reference grade III)	V	-15.22 [-30.2;-0.2]	0.046	10.76 [-31.2;52.7]	0.615
Hypertension		-6.48 [-11.6;-1.4]	0.013	-8.34 [-22.1;5.3]	0.233
eGFR at biopsy		0.27 [0.2;0.3]	<0.001	-0.11 [-0.3;0.0]	0.123
UPUC at biopsy		0.00 [0.0;0.0]	0.049	-0.00 [-0.0;0.0]	0.607
Mycophenolate mofetil		-6.91 [-13.6;-0.2]	0.043	50.27 [31.7;68.8]	<0.001
Calcineurin inhibitor		-10.42 [-16.9;-3.9]	0.002	37.30 [19.4;55.2]	<0.001
Rituximab		-26.28 [-47.2;-5.4]	0.014	-2.46 [-64.0;59.0]	0.937
Time onset to treatment		-0.01 [-0.0;-0.0]	0.001	0.02 [-0.0;0.0]	0.451

eGFR estimated glomerular filtration rate, UPUC urinary protein/creatinine ratio, CI Confidence interval 95%, ISKDC international study of kidney disease in children

Table 5: Outcome at last follow-up in the different severity groups defined at onset analysed with multivariate analysis: Patients with biopsy score MEST C included, only treatments are listed below. a) influence on eGFR, b) on UPUC at last follow up.

a)

Influencing factor on eGFR at latest follow up	eGFR<60 (n=76)		eGFR<90 (n=176)		NS (n=82)		Albumin <35 g/l at onset (n=279)	
	Coefficient [CI]	p	Coefficient [CI]	p	Coefficient [CI]	p	Coefficient [CI]	P
ACE- inhibition	-22.49 [-43.1;-1.9]	0.033	-8.61 [-19.4;2.2]	0.116	6.56 [-19.7;32.8]	0.619	-3.77 [-13.8;6.2]	0.458
Steroids	-0.44 [-64.8;63.9]	0.989	-2.17 [-18.1;13.8]	0.788	8.86 [-49.1;66.9]	0.761	-2.40 [-17.2;12.4]	0.749
MMF	-2.29 [-21.3;16.7]	0.809	-8.04 [-18.6;2.5]	0.134	-8.58 [-30.1;13.0]	0.429	-11.31 [-20.7;-1.9]	0.019
Azathioprine	1.50 [-22.2;25.2]	0.899	-5.79 [-17.1;5.5]	0.314	-10.13 [-29.1;8.8]	0.288	-12.31 [-21.7;2.9]	0.010
Cyclophosphamide	-8.50 [-24.9;7.9]	0.302	-9.06 [-20.9;2.7]	0.132	-0.28 [-18.5;17.9]	0.976	-2.52 [-11.4;6.3]	0.575
Calcineurin inhibitor	1.36 [-22.3;25.0]	0.909	-24.95 [-39.9;-10.0]	0.001	0.71 [-22.4;23.9]	0.951	-16.37 [-27.8;-4.9]	0.005
Immunoglobulins			14.37 [-37.8;66.5]	0.587	-27.61 [-91.9;36.7]	0.393	-5.04 [-46.3;36.2]	0.810
Rituximab	-21.62 [-75.5;32.2]	0.424	-53.08 [-82.1;-24.1]	<0.001			-41.92 [-75.6;-8.2]	0.015
Plasmapheresis	-16.28 [-49.8;17.3]	0.334	-8.48 [-30.9;13.9]	0.456	-5.27 [-45.0;34.5]	0.792	0.84 [-20.3;22.0]	0.938
Anticoagulation	-41.63 [-65.0;-18.3]	<0.001	-21.85 [-38.9;-4.8]	0.013	-29.19 [-52.7;-5.7]	0.016	-7.04 [-21.1;7.0]	0.325

b)

Influencing factor on UPUC at latest follow up	eGFR<60 (n=75)		eGFR<90 (n=179)		NS (n=86)		Albumin <35 g/l at onset (n=292)	
	Coefficient [CI]	p	Coefficient [CI]	p	Coefficient [CI]	P	Coefficient [CI]	p
ACE- inhibition	22.61 [-44.8;90.0]	0.504	15.58 [-16.5;47.6]	0.339	15.53 [-37.6;68.5]	0.560	9.21 [-22.9;41.3]	0.572
Steroids	-4.82 [-223.7;214.0]	0.965	-2.16 [-48.7;44.3]	0.927	35.10 [-92.8;163.0]	0.585	-6.13 [-54.7;42.4]	0.804
MMF	-38.52 [-101.8;24.7]	0.227	15.97 [-16.2;48.1]	0.328	25.31 [-23.1;73.7]	0.300	32.47 [1.1;63.8]	0.042

Azathioprine	24.35 [-51.1;99.8]	0.520	5.06 [-28.1;38.2]	0.763	44.09 [4.6;83.6]	0.029	34.02 [4.1;64.0]	0.026
Cyclophosphamide	30.33 [-23.1;83.8]	0.260	48.60 [13.1;84.0]	0.008	30.58 [-10.0;71.2]	0.137	25.54 [-4.3;55.3]	0.093
Calcineurin inhibitor	7.68 [-73.1;88.4]	0.849	-20.00 [-69.2;29.2]	0.423	29.29 [-21.5;80.1]	0.253	61.19 [23.6;98.8]	0.002
Immunoglobulins			3.51 [-149.6;156.6]	0.964	-19.53 [-158.4;119.3]	0.780	-105.96 [-248.0;36.1]	0.143
Rituximab	235.09 [62.4;407.7]	0.009	76.97 [-27.8;181.8]	0.149			166.30 [28.3;304.3]	0.018
Plasmapheresis	68.59 [-55.2;192.4]	0.271	-9.87 [-90.2;70.5]	0.809	-2.54 [-109.6;104.5]	0.962	2.73 [-77.2;82.6]	0.946
Anticoagulation	-22.54 [-98.0;52.9]	0.551	-14.92 [-66.5;36.7]	0.569	-2.86 [-52.0;46.2]	0.908	-35.56 [-77.3;6.2]	0.095

CI confidence interval 95%, ACE angiotensin converting enzyme, eGFR estimated glomerular filtration rate, MMF mycophenolate mofetil, NS nephrotic syndrome (albumin <25g/l and UPUC >200mg/mmol), UPUC urinary protein/creatinine ratio

Table 6: Comparison of different treatments in patients with different severity at onset and at last follow up

eGFR at onset (ml/min/1.73m^2)	eGFR at last follow up (ml/min/1.73m^2)	MMF	Azathioprine	Cyclophosphamide	CNI	p
>90	>90	24 (66.7%)	51 (92.7%)	48 (92.3%)	33 (76.7%)	0.001
	<90	12 (33.3%)	4 (7.3%)	4 (7.7%)	10 (23.3%)	
<90	>90	12 (48%)	10 (62.5%)	18 (58.1%)	5 (41.7%)	0.624
	<90	13 (52%)	6 (37.5%)	13 (41.9%)	7 (58.3%)	

UPUC at last follow up (mg/mmol)	UPUC at last follow up (mg/mmol)	MMF	Azathioprine	Cyclophosphamide	CNI	p
<200	<20	4 (30.8%)	20 (69.0%)	15 (83.3%)	10 (66.7%)	0.022
	>20	9 (69.2%)	9 (31.0%)	3 (16.7%)	5 (33.3%)	
>200	<20	26 (56.5%)	34 (65.4%)	42 (57.5%)	27 (67.5%)	0.594
	>20	20 (43.5%)	18 (34.6%)	31 (42.5%)	13 (32.5%)	

eGFR estimated glomerular filtration rate, MMF mycophenolate mofetil, CNI calcineurin inhibitor, UPUC urinary protein/creatinine ratio

Figure legends

Figure 1 A) eGFR and **B)** proteinuria at onset and last follow-up in the different treatment groups

eGFR estimated glomerular filtration rate, UPUC urinary protein/creatinine ratio

Supplementary Material

Supplementary Figure S1: Subgroups depending on the severity at onset and outcome for eGFR and UPUC

Supplementary Table S1: Participating centres and number of patients included

Supplementary Table S2: Baseline data of different treatment groups

Supplementary Table S3: Factors at biopsy influencing eGFR and UPUC at last follow-up**:** Univariate analysis of all factors, n number of patients included

Supplementary Table S4: Baseline data in the different severity groups