UNIVERSITY OF LIVERPOOL

Understanding disease activity in children with IgA vasculitis

Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Master of Philosophy by Julien Gilbert Marro

[July 2022]

Declaration

I declare that this thesis and the research upon which it is based is the result of my own work. Wherever I have incorporated the work of other, it has clearly been stated.

This work has not already been accepted in substance for any degree, nor is it being concurrently submitted in candidature for any degree in this or another University.

Julien Gilbert Marro

[July 2022]

Table of contents

Abstract

Introduction

IgA vasculitis (IgAV, formerly Henoch Schönlein purpura – HSP) is the most common vasculitis encountered by paediatricians. Despite an excellent outcome in the vast majority of children, a small subgroup will suffer from a prolonged disease course and renal involvement (IgAV nephritis – IgAVN) remains a concern with 1-2% developing chronic kidney disease 5. An improved understanding of the disease pathophysiology alongside standardisation of care is required to improve disease outcomes.

Aim

The aim of this thesis was to improve the understanding of disease activity in paediatric IgAV.

Material and methods

Initially, this thesis retrospectively described the clinical characteristics and factors associated with children with recurrent/persisting disease and a systematic literature review was performed to identify definitions of recurrent and persisting IgAV in order to propose standardised definitions. Subsequently, this thesis used enzyme-linked immunoassays (ELISA) on a cohort of 47 children with IgAV and 12 healthy controls (HCs) to identify differences in the urinary concentrations of immunoglobulin A (IgA) and complement C5a. Finally, proteome profilers array kits were used to evaluate the variations in the urinary proteome between patients with and without nephritis by semi-quantitatively assessing 124 key proteins of inflammation in children with IgAV and HCs.

Results

A total of 13 children were included in the case series: (9 recurrent, 4 persisting). Joint involvement was the main reason prompting referral, the median time from initial presentation to diagnosis was 18.4 months (range [5.3-150.8]) and to treatment with disease-modifying anti-rheumatic drugs was 24.1 months [1.8-95.4]. The systematic literature review identified 40 studies, allowing a proposed definition of recurrent IgAV ('a new onset of typical purpura alongside any other characteristic signs of IgAV -as per EULAR/PRINTO/PReS- at a time 4 weeks after the complete resolution of previous symptoms') and persisting IgAV ('typical purpura alongside any other characteristic signs of IgAV -as per EULAR/PRINTO/PReS- lasting over 4 weeks').

The ELISA data revealed that urinary concentrations of IgA and C5a were statistically significantly increased in patients with nephritis -IgAVN group- (IgA-205.0µg/mmol [51.8-850.1]; C5a-66.4ng/mmol [12.5-345.1]) compared to patients without nephritis -IgAVwoN group- (IgA-p=0.006; C5a-p=0.006) and HCs (IgA-p<0.001; C5a-p=0.001). Both markers were individually excellent at

distinguishing IgAV patients with and without nephritis (IgA–AUC=0.811, *p*=0.002; C5a–AUC=0.826, *p*=0.001).

The proteome profilers identified 20 urinary proteins that were significantly increased in IgAVN compared to IgAVwoN. The largest fold-changes were reported for B-cell activating factor, Cripto-1, sex-hormone binding globulin, angiotensinogen and apolipoprotein A1. The urinary levels of complement components C5/C5a and factor D were also statistically significantly increased in IgAVN.

Conclusion

This thesis investigated some aspects of complex disease activity in paediatric IgAV. Initially, in children with a recurrent and/or prolonged disease course, then the discovery of urinary markers of kidney inflammation provided novel insight into the pathophysiology of IgAVN and potential therapeutic targets. Further large longitudinal studies, randomised clinical trials and standardised clinical guidelines are urgently needed to improve outcomes in this disease.

Acknowledgements

First, words cannot express my gratitude to my supervisor, Dr Louise Oni, for her unparalleled support and guidance throughout the year. Her dedication to my academic development has gone above and beyond the expectations of an MPhil supervisor and she has provided me with the most incredible opportunities. She has been an outstanding mentor and role model: I will be sure to take what I have learnt from her throughout my career.

Secondly, to Dr Andrew Chetwynd, for his amazing supervision throughout the year, for sharing with me his extensive knowledge and for his valuable constructive feedback. He has remained so approachable and incredibly patient. To Dr Rachael Wright, for her supervision and help at the start of the year, and for staying approachable even after leaving the laboratory group.

To Catherine McBurney, Silothabo Dliso, Jessica Tiffins, all of the Clinical Research Facility staff and all of the EATC4Children group, for making me feel so welcome and treating me as a valued member of their teams from day one. The things I have learned from each and every one of them are priceless and have not only contributed to this thesis and its outputs but will be invaluable to my future career. It has been a real pleasure to work alongside such inspiring clinicians, scientists and technicians.

Next, I would like to thank the people and co-authors who contributed to the work included in this thesis: Sarah Northey and Rachel Corkhill for conducting the C5a ELISAs; Sam Edwards for helping to conduct the serum IgA ELISAs; Chloe Williams for helping to identify the cohort for the case series.

Then, I would like to express my sincere gratitude to the FAIR charity for making this research possible through their generous financial support, and to the BAPN, whose financial support allowed me to travel to Slovenia for a conference.

To my friends and housemates for always making time to listen to me after a long day and for their eternal optimism. To my MPhil and library buddy, Lily Jones, for her moral support and her reassuring words in the slight moments of panic. To my mum, for always believing in me and supporting me throughout my studies.

Finally, and more importantly, I am most grateful to the children and young people who participated in the IgAV study and their parents/carers. None of this work would have been possible without them and we will continue to strive to improve the health and wellbeing of children and young people living with autoimmune diseases.

Outputs arising from this MPhil

Publications

Marro, J.; Chetwynd, A.J.; Wright, R.D *et al*. Urinary Protein Array Analysis to Identify Key Inflammatory Markers in Children with IgA Vasculitis Nephritis. *Children* (2022). https://doi.org/10.3390/children9050622 (**Appendix 1**).

Rachael D Wright; Julien Marro; Sarah J Northey; *et al*. Urinary complement proteins are increased in children with IgA vasculitis (Henoch-Schönlein purpura) nephritis. Submitted to *Pediatric Nephrology* on May 4th, 2022.

Andrew J Chetwynd, Julien Marro, Laura Whitty *et al*. How are micro-sampling and home testing kits perceived by children and young people? Submitted to *Health Expectations* on June 6th, 2022.

Presentations

Julien Marro, Rachael D. Wright, Andrew J. Chetwynd *et al*. Oral presentation at the BAPN Winter Meeting (January 2022). Urinary IgA and C5a are indicators of renal involvement in children with IgA Vasculitis.

Julien Marro, Chloe Williams, Louise Wadden *et al*. Oral presentation & poster at the International ANCA and Vasculitis Workshop in Dublin (April 2022). A case series on recurrent and persisting IgA Vasculitis in Children.

Julien Marro, Rachael D. Wright, Andy J. Chetwynd *et al*. Poster at the International ANCA and Vasculitis Workshop in Dublin (April 2022). Urinary IgA and C5a are indicators of renal involvement in children with IgA Vasculitis.

Julien Marro, Andrew J. Chetwynd, Rachael D. Wright *et al*. Oral presentation at the Cheshire and Mersey Academic Nephrology & Transplant Showcase (May 2022). Urinary Protein Array Analysis to Identify Key Inflammatory Markers in Children with IgA Vasculitis Nephritis.

Julien Marro, Andrew J. Chetwynd, Rachael D. Wright *et al*. Poster at the 54th annual ESPN Meeting (Slovenia, June 2022). Urinary Protein Array Analysis to Identify Key Inflammatory Markers in Children with IgA Vasculitis Nephritis.

Julien Marro, Andrew J. Chetwynd, Rachael D. Wright *et al*. Oral presentation at the RCPCH Conference (Liverpool, June 2022). Urinary Protein Array Analysis to Identify Key Inflammatory Markers in Children with IgA Vasculitis Nephritis.

Julien Marro, Chloe Williams, Louise Wadden *et al*. Poster at RCPCH Conference (Liverpool, June 2022). A case series on recurrent and persisting IgA Vasculitis in Children.

Prices and awards

- 3rd best oral presentation (British Association for Paediatric Nephrology, 2022)
- International Vasculitis and ANCA Workshop registration bursary (2022)
- BAPN travel award (British Association for Paediatric Nephrology, 2022)

COVID-19 disruptions

During this year, the course of the COVID-19 pandemic has been easing and therefore, the Institute in the Park laboratory and offices did not close. In addition, recruitment to all studies at Alder Hey remained open. However, COVID-19 still has had some minor indirect disruptions on my MPhil year. Most meetings were held online, and the BAPN Winter Meeting, which was my first conference, had to be held completely virtually due to concerns regarding the emergence of Omicron. Similarly, the shift towards the use of “e-Posters”, even in face-to-face conferences that I was lucky to attend, meant fewer opportunities to interact and network during poster viewing sessions. In terms of the recruitment to the study, patients were overall less likely to attend their follow-up appointments and it was more challenging to recruit patients and healthy controls at times when clinicians were already under pressure due to the pandemic.

List of tables

List of figures

List of abbreviations

AAV	ANCA-associated vasculitis
ACE	Angiotensin-converting enzyme
ACR	American College of Rheumatology
ADCC	Antibody-dependent cell-mediated cytotoxicity
AECAs	Anti-endothelial cell antibodies
AGT	Angiotensinogen
AJC	Andrew J. Chetwynd
ANA	Antinuclear antibodies
ANCA	Antineutrophil cytoplasmic antibodies
ApoA1	Apolipoprotein A1
ARB	Angiotensin II receptor blocker
ASO	Antistreptolysin O
AUC	Area under the curve
BAFF	B-cell activating factor
BP	Blood pressure
BVAS	Birmingham Vasculitis Activity Score
CASP	Critical Appraisal Skills Program
CD40L	Cluster of differentiation 40 ligand
CDC	Complement mediated toxicity
CENTRAL	Cochrane Central Register of Controlled Trials
CFB	Complement factor B
CFD	Complement factor D
CKD	Chronic kidney disease
Cr	Creatinine
CRF	Case report form
CS	Corticosteroids
CW	Chloe Williams
CXCL1	CXC motif chemokine 1
CXCL16	CXC motif chemokine 16
DMARDs	Disease-modifying antirheumatic drugs
eGFR	Estimated glomerular filtration rate
EGF-R	Epithelial growth factor receptor
ELISA	Enzyme-linked immunosorbent assay
ENT	Ear, nose and throat
ESR	Erythrocyte sedimentation rate

EULAR	European Alliance of Associations for Rheumatology
FC	Fold-change
FcαRI	Fc alpha receptor (CD89)
FMF	Familial Mediterranean fever
GalNac	N-acetylgalactosamine
GDG	Guideline development group
Gd-IgA1	Galactose-deficient IgA1
Gd-IgA1 CICs	Gd-IgA1 containing immune complexes
GH	Growth hormone
GI	Gastrointestinal
GIGA-kids study	Genomics of IgA-related disorders in kids Study
GP	General practitioner
GWAS	Genomic wide association studies
HCRW	Health and Care Research Wales
HCs	Healthy controls
HGF	Human growth factor
HLA	Human leukocyte antigens
HRA	Health research authority
HSP	Henoch Schönlein purpura
HTA	Human tissue act
ICAM-1	Intracellular adhesion molecule 1
ICD	International Classification of Diseases
IF	Immunofluorescence
Ig	Immunoglobulin
IgAN	IgA nephropathy
IgAV	IgA vasculitis
IgA-VAS	IgA Vasculitis disease Activity Score
IgAVN	IgA vasculitis with nephritis
IgAVwoN	IgA vasculitis without nephritis
IGFBP-3	Insulin-like growth factor-binding protein 3
IL	Interleukin
ISKDC	International Study of Kidney Disease in Children
IT	Information technology
IV	Intravenous
IVIG	Intravenous immunoglobulins
JM	Julien Marro
KDIGO	Kidney Disease: Improving Global Outcomes

KIM-1	Kidney injury molecule 1
L-FABP	Liver-type fatty acid binding protein
LIF	Leukaemia inhibitory factor
LO	Louise Oni
LTB4	Leukotriene B4
MAC	Membrane attack complex (C56b-9)
MASPs	MBL-associated serine proteases
MBL	Mannose-binding lectin
MCP-1	Monocyte chemoattractant protein-1
MDU	Medical day case unit
MEST-C	Oxford classification of IgA nephropathy
MMF	Mycophenolate mofetil
MMP-9	Matrix metalloproteinase 9
NAG	N-acetyl-β-glucosaminidase
NETS	Neutrophil extracellular traps
NGAL	Neutrophil gelatinase-associated lipocalin
NHS	National Health Service
NICE	National Institute for Health and Care Excellence
NIHR	National Institute for Health and Care Research
NSAIDs	Non-steroidal anti-inflammatory drugs
P	Properdin
PBS + 1% PSA	Phosphate buffered saline + 1% bovine serum albumin
PC	Principal component
PMBC	Peripheral blood mononuclear blood cells
PReS	Paediatric Rheumatology European Society
PRINTO	Paediatric Rheumatology INternational Trials Organisation
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PVAS	Paediatric Vasculitis Activity Score
RAAS	Renin-angiotensin-aldosterone system
RC	Rachel Corkhill
RCPCH	Royal College of Paediatrics and Child Health
RCT	Randomised controlled trial
ROC	Receiver operating characteristic
ROS	Reactive oxygen species
RT	Room temperature
sCD89	soluble IgA Fc alpha receptor
SD	Standard deviation

SE	Sam Edwards
SHARE	Single Hub and Access point for paediatric Rheumatology in Europe
SHBG	Sex hormone binding globulin
SLE	Systemic lupus erythematosus
SN	Sarah Northey
SP	Streptavidin-peroxidase
SQC	Semiquantitative classification
ST	Specialist registrar
ST2	Suppression of tumorigenicity 2
Streptavidin-HRP	Horseradish peroxidase-conjugated Streptavidin
TfR	Transferrin receptor
TG2	Transglutaminase 2
TNF	Tumour necrosis factor
UACR	Urinary albumin to creatinine ratio
UAGT	Urinary angiotensinogen
UK	United-Kingdom
URTIs	Upper respiratory tract infections
WBC	White blood cells
ΔOD	Difference in optical density

1. Introduction

1.1. IgA vasculitis in children

Immunoglobulin A vasculitis (IgA vasculitis -IgAV-, formerly Henoch Schönlein purpura -HSP-) is an immune complex-mediated vasculitis affecting the small blood vessels. It is the vasculitis most frequently encountered by paediatricians (1) and often presents acutely with a purpuric rash, arthritis/arthralgia, gastrointestinal symptoms and/or kidney involvement (2). Henoch-Schönlein purpura was first described by Heberden in 1806. The association of purpura and joint pain was reported by Schönlein in 1837, who named the disease "peliosis rheumatica." In 1874, four cases of children with skin lesions, colicky abdominal pain, gastrointestinal bleeding and joint pain were described by Henoch and in 1899, he described for the first-time renal involvement in HSP (3). In 2012, the eponym "Henoch-Schönlein purpura" was replaced by "IgA vasculitis" in an attempt to standardise nomenclature to better reflect the pathophysiology of this disease (2), although the term HSP is still widely used in clinical practice. This thesis will focus on paediatric IgA vasculitis.

1.1.1. Epidemiology

Despite being the most common vasculitis in children, IgAV is rare and the annual incidence rates have been reported to range from 3 to 27.2 per 100.000 children. Peak incidence is 4-7 years old, with 90% of patients presenting aged less than 10 years (4). It is less common in teenagers and rare in adults (5, 6). Children older than 10 years old at presentation are at an increased risk of developing renal involvement (7) and increasing age at onset may be a risk factor for a poorer prognosis, which includes progression to chronic kidney disease stage 5 (CKD 5) and relapsing/persisting IgAV (8-12). The disease has a slight male predominance with a male: female ratio of 1.5:1 (1), although this can vary across studies.

Different incidence rates have been reported for different ethnic populations, yet, evidence is lacking to ascertain any true ethnic variation. The highest incidence rate was reported in Korea at 55.9 per 100,000 children per year (13) but other studies of Asian populations reported lower incidence rates ranging from 9.6 to 12.9 (14, 15). Perhaps, the most robust study to date that estimated the annual incidence of IgAV in children from different ethnic origins was conducted in Birmingham (West Midlands, UK) in 2002. In this study, children of Asian or Caucasian ethnicity had the highest annual incidence rate of IgAV at 20.4 and 17.8/100,000 children respectively whereas children of Black ethnicity showed a significantly lower incidence rate at 6.2 / 100,000 children per year (1). Likewise, a French study also found a lower incidence in children from a North-African background (16). In Europe, the annual incidence rate is around 20 / 100,000 children (5, 16-18) and in the UK, the most recent study reported 27.2 per 100,000 children per year (6).

1.1.2. Aetiology

The cause of IgA vasculitis is currently unknown; however, the disease process is likely to be triggered by environmental factors in a genetically predisposed host (19).

1.1.2.1. *Genetic predisposition*

Genetics is thought to play a key role in not only IgAV susceptibility but also its severity (19). The first cases of familial IgAV were described in 1989 (20) and since then, several studies have shown that aberrant glycosylation of IgA1 (i.e., lacking galactose in its hinge region, see **1.1.3.1**) may be inherited in IgAV nephritis (IgAVN) (21, 22). However, familial clustering of IgAV remains rare (23, 24) and IgAV heritability has not been proven yet (23).

Only two small genomic wide association studies (GWAS) (including 285 IgAV patients and 1,006 controls (25); 46 paediatric patients with IgAV and 49 diseased-control paediatric patients with an inflammatory-bowel disease (26), respectively) have been conducted so far for IgAV. They showed a strong association between disease susceptibility and human leukocyte antigen (HLA) class II alleles, especially with *HLA-DRB1* (25, 26). In addition to HLA class II, a relationship with HLA class I alleles was reported, although not as strong as what was found for the former, and the authors noted that susceptibility to most vasculitides was linked to the HLA region (27).

The most recent comprehensive review of the genetic factors involved in the pathogenesis of IgAV was produced by Lopez-Meijas and colleagues in 2018 (27) and highlighted gene polymorphisms located outside of the HLA region. Those included genes encoding for cytokines, chemokines, adhesions molecules, alongside those related to T-cells, aberrant glycosylation of IgA1, nitric oxide production, neo-angiogenesis, renin-angiotensin system and lipid, pyrin and homocysteine metabolism, which may be implicated in both the susceptibility and severity of the disease (27). Furthermore, IL-1, IL-8, and ACE polymorphisms were associated with the most severe renal phenotype (19, 27). Finally, IgAV can occur in 2.7-7% of patients with familial Mediterranean fever (FMF) (28) and some studies have highlighted the potential association of *MEFV* gene mutations with IgAV susceptibility, although results are inconsistent across studies (27).

Most of those studies, apart from the GWAS, were small underpowered studies using limited genetic methods (29). In light of those issues, the GIGA-kids study (Genomics of IgA-related disorders in kids, www.gigakids.org) was established to investigate the genomic and genetic aspects of IgAV, IgAVN and IgA nephropathy in children (23). Recruitment is still ongoing for this study and the first results are expected by the end of 2022. In the UK, patients with biopsy-proven IgA nephropathy secondary to IgAV are also eligible to take part in the National Institute for Health and Care Research

(NIHR) BioResources Rare Diseases IgA nephropathy cohort, which aims to develop a national bioresource of participants with rare diseases mainly for genotyping purposes (30).

1.1.2.2. *Environmental factors*

In children, the disease demonstrates a seasonal pattern with an increased incidence during the spring and winter and fewer cases occurring in the summer. This seasonal tendency was shown to follow the epidemic patterns of various viruses such as influenza, rotavirus, respiratory syncytial virus as well as norovirus (31, 32) and supports the theory that viral upper respiratory tract infections (URTIs) are triggers for this disease. Other infectious agents have also been associated with IgAV onset, especially *group A Streptococcus*, *Staphylococcus Aureus*, *Helicobacter Pylori*, varicella-zoster virus, hepatitis virus, human immunodeficiency virus, cytomegalovirus, *Clostridium Difficile* (33) and more recently, COVID-19 (34). Since IgA is secreted from mucosal surfaces, it was proposed that the ear, nose and throat system (ENT) may play a key role in the interplay between infection and abnormal IgA secretion leading to IgAV (19). In addition, oral and ENT diseases are frequent in paediatric patients with IgAV and those with chronic tonsillitis were found to be at increased risk of recurrent disease and renal involvement (35).

Some evidence suggests that IgAV may be triggered by certain medications and historically, penicillin, cefaclor, minocycline, hydralazine and phenytoin were thought to increase the risk of developing IgAV (36, 37). A recent French pharmacovigilance study identified several antibiotics (beta-lactamines, fluoroquinolones and macrolides) and TNF-α blockers as being associated with IgAV occurrence (38). However, as bacterial infections can induce IgAV, it could be a potential confounder and a case-control Italian study did not find any association between medications and the risk of developing IgAV (39). Similarly, for vaccines, findings are conflicting. Increasing risk of developing IgAV was significantly associated with measles-mumps-rubella (39) and all types of vaccines (38) in the case-control study and the French pharmacovigilance study respectively. On the other hand, a larger European multi-centre study found that vaccines most commonly administered to children did not significantly increase the short-term risk of IgAV onset (40). Nonetheless, Rasmussen *et al*. proposed that vaccines could trigger and promote the onset of IgAV by mimicking the immune response against pathogens (38).

Spatial clustering of IgAV and IgAVN has been reported by one Croatian study. Particularly, linear clustering of IgAVN in the eastern part of Croatia was shown to follow the Drava and Danube rivers (41) in a similar pattern that of Balkan endemic nephropathy, which was associated with daily exposure to ochratoxin A, a common mycotoxin, in wheat and corn from this region (42). Sapina and

colleagues therefore hypothesised that IgAV and IgAVN clusters may appear in areas of substantial genetic and environmental factors overlap (41).

1.1.3. Pathophysiology

As its name suggests, IgAV is mainly driven by IgA1 immune deposits (19). IgA is the most abundant antibody present in the mucosa and plays a key role in host-pathogen first-line defence despite being continuously exposed to antigens, food and commensal microorganisms. Hence, normally functioning IgA keeps a tight balance between protecting its host against harmful pathogens while tolerating food and commensal flora (43). IgA has two subtypes: IgA1 and IgA2 and can be divided into its monomeric forms in serum compared to forming dimers in as secretory IgA. IgA1 structurally differs from IgA2 by its O-linked glycan-rich hinge region (44), which contains three to six O-linked glycan sites.

The pathophysiology of IgAV is complex and it may differ between patients with different phenotypes.

1.1.3.1. *Galactose deficient IgA1 and its autoantibodies*

Healthy IgA1 is glycosylated with N-acetylgalactosamine (GalNac), galactose and sialic acid whereas in IgAVN, the O-glycans in the hinge region are aberrantly glycosylated (i.e., lack galactose) (**Figure 1.1**). Higher serum levels of galactose-deficient IgA1 (gd-IgA1) alongside gd-IgA1 dominant deposits in the kidney, skin and GI tract tissues are found in patients with IgAV (44). In addition, higher serum gd-IgA1 levels are associated with a higher risk of renal involvement in these patients. The production of gd-IgA1 is thought to be the leading phenomenon triggering the disease, hence it is considered to be the first hit of its pathophysiology. However, it is unknown what is causing the production of the gd-IgA1. This could be linked to polymorphisms in genes coding for enzymes involved in O-glycosylation of IgA1 by B-cells or dysregulation of cytokines, such as IL-6 and IL-8, which also participate in the regulation of these enzymes. As discussed above, IgAV is often triggered by a URTI suggesting mucosal antigens play a key role in the disease onset. Activated B-cells can produce gd-IgA1 in mucosa-associated lymphoid tissue in a T-cell dependent or independent manner, which involves the interaction between B-cell, dendritic cells, and Toll-like receptor pathway (44). However, although the role of gd-IgA1 in IgAVN is well recognised, its role in systemic disease and the vasculitis itself in patients without nephritis is more controversial.

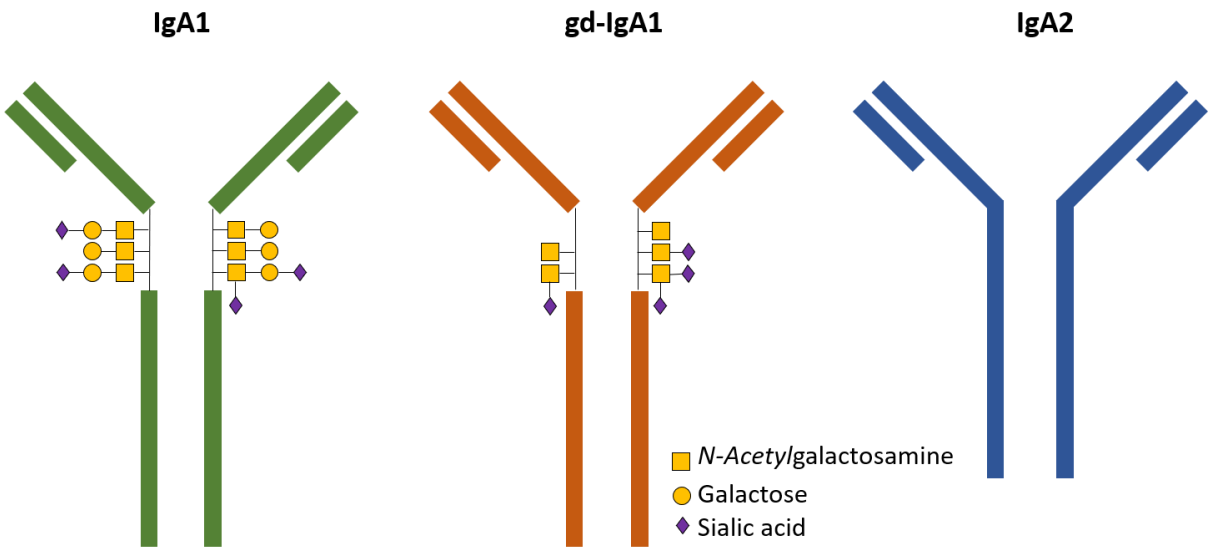


***Figure 1.1**: The different forms of IgA. IgA1 hinge region contains three to six O-linked glycan sites and healthy IgA1 is glycosylated with N-acetylgalactosamine, galactose and sialic acid whereas galactose-deficient IgA1 (gd-IgA1) lacks galactose in its hinge region. IgA2 does not have a hinge region and does not possess O-glycans. Both IgA1 and IgA2 have N-glycans (not shown here). Adapted from Heineke et al. (45).*

GalNac residues found in the hinge regions of gd-IgA1 or mucosal antigens mimicking the structure of gd-IgA1 can then be recognised as foreign by the host and induce a humoral response, resulting in the production of anti-gd-IgA IgG autoantibodies (44).

1.1.3.2. *Formation of pathogenic gd-IgA1 immune complexes*

Gd-IgA1 alone is not sufficient to cause the disease and family members of IgAV patients can have elevated serum gd-IgA1 levels without any clinical symptoms, hence the formation of gd-IgA1-containing immune complexes (gd-IgA1 CICs) is thought to be essential to the disease onset and progression. Gd-IgA1 can self-aggregate (forming poly-IgA1 aggregates) or binds to IgG autoantibodies (44). In addition, gd-IgA1 can form immune complexes with soluble IgA Fc alpha receptor (sCD89), whose levels were shown to correlate with disease severity and progression (45).

1.1.3.3. *Deposition of gd-IgA1-containing immune complexes*

Small circulating immune complexes are usually cleared by hepatocytes in the liver whereas gd-IgA1 CICs are large and cannot be cleared normally. They therefore accumulate and can deposit in the small vessel walls, causing subsequent inflammation. However, the intensity of gd-IgA1-CICs deposition in the kidney and skin does not correlate with the serum levels of gd-IgA1 (46), which suggests that immune complexes deposition is influenced by other factors than their size or quantity (44).

1.1.3.3.1. In IgAVN

In the kidneys, gd-IgA1 CICs, especially gd-IgA1-sCD89 complexes, can activate mesangial cells through the transferrin receptor (TfR), which has a higher affinity for gd-IgA1 compared to normally

glycosylated IgA1 (47). This subsequently induce transglutaminase 2 (TG2) on mesangial surface leading to upregulation of TfR expression. This is thought to initiate a positive feedback loop resulting in enhanced immune complex deposition as well as further mesangial cell activation. This leads to mesangial cell proliferation, complement activation, production of pro-inflammatory cytokines and chemokines (especially IL-6, IL-8, TNFs and MCP-1), and apoptosis of podocytes and tubular epithelial cells, all of which contributes to the recruitment of inflammatory cells and ultimately renal inflammation (**Figure 1.2**).

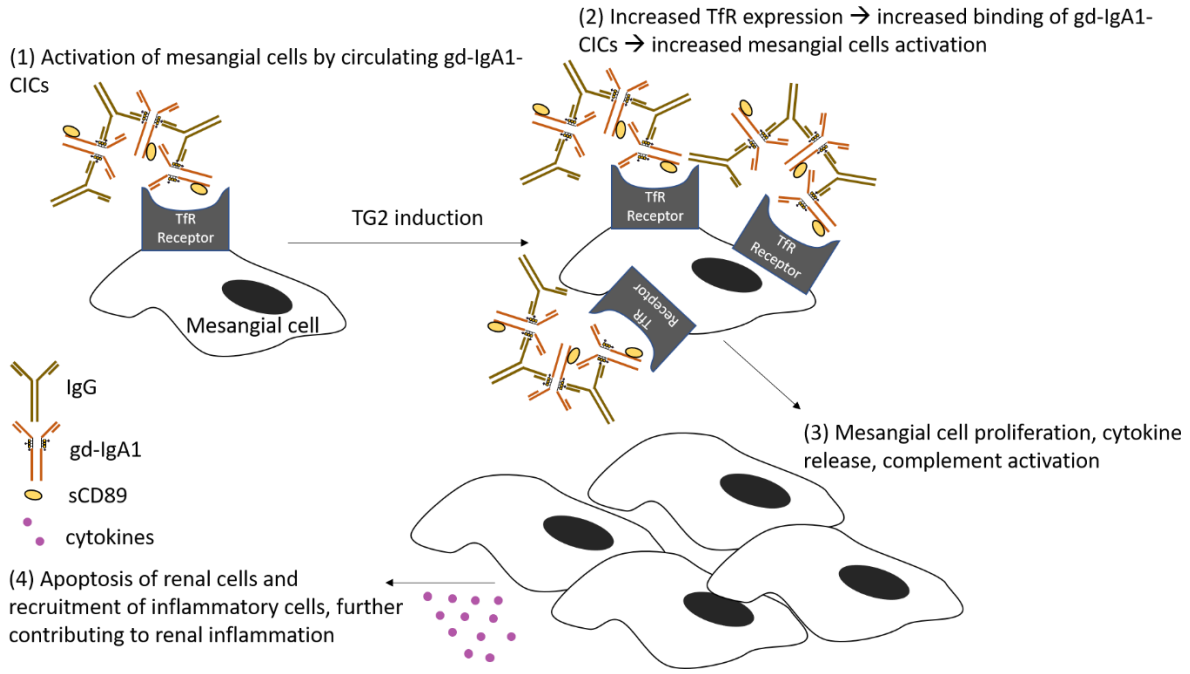


***Figure 1.2**: Deposition of gd-IgA1 immune complexes in the kidneys, which results in kidney inflammation. Gd-IgA1-CICs: galactose deficient IgA1 containing immune complexes; TfR: transferrin; TG2: transglutaminase 2; sCD89: soluble IgA Fc alpha receptor. Adapted from Heineke et al (45).*

1.1.3.3.2. In extra-renal manifestations

It is unclear whether gd-IgA1-CICs play a role in the extra-renal components of IgAV. Several studies have found no significant difference in serum gd-IgA1 levels between healthy controls and patients with IgAV without nephritis. In addition, IgG-containing immune complexes were reported to be absent in patients with no renal involvement as opposed to IgAVN (44). Yet, a recent study by Neufeld and colleagues found cutaneous gd-IgA1 deposition in adult patients with both skin-limited (n = 12) and systemic (n = 4) IgAV using KM55 staining. However, in this study, gd-IgA1 serum levels were significantly higher in IgAVN patients compared to those with skin-limited IgAV, and no significant difference was observed between the latter group and the healthy controls. The authors proposed that perivascular gd-IgA1 deposition was required for both systemic and skin-limited IgAV and that high gd-IgA1 levels in some patients with systemic IgAV may suggest a dose-dependent effect (48).

1.1.3.4. *The potential role of anti-endothelial cell antibodies (AECAs)*

The specific autoantibodies to which IgA1 binds in extra-renal IgAV remains unknown, but more recent evidence suggests anti-endothelial cell antibodies (AECAs) may be involved. AECAs are a group of antibodies targeted to endothelial cells and have been described in almost all primary systemic vasculitides, including in IgAV (45, 49). Although AECAs are most commonly IgG antibodies, IgM and IgA AECAs have been described and in IgAV, AECAs are exclusively of the IgA isotype (48). Their role in IgAV pathogenesis has been proposed by Legendre *et al*. and Heineke *et al.*, this is summarised in **Figure 1.3** (45, 49). Pathogenic agents may have a similar antigenic structure to human vessel walls and infection with such microorganisms could result in the production of cross-reactive IgA1-AECAs. Binding of IgA1-AECAs to autoantigens on endothelial cells, enhanced by TNF-α, may result in IL-8 release from endothelial cells. IL-8 can induce neutrophil migration and the interaction between IgA1-AECAs and FcαRI on neutrophils induce leukotriene B4 (LTB4) release, which results in a positive feedback loop further recruiting neutrophils. In addition, ACEAs may upregulate the expression of adhesion molecules by endothelial cells, such as E-selectin or intercellular adhesion molecule 1 (ICAM-1). Vascular damage may therefore be promoted by IgA through antibody-dependent cellular cytotoxicity (ADCC), reactive oxygen species (ROS) production, complement dependent toxicity and neutrophils extracellular traps (NETs) formation. This process could be responsible for the characteristic leukocytoclastic vasculitis (i.e. perivascular neutrophils infiltration) seen in IgAV (45, 49).

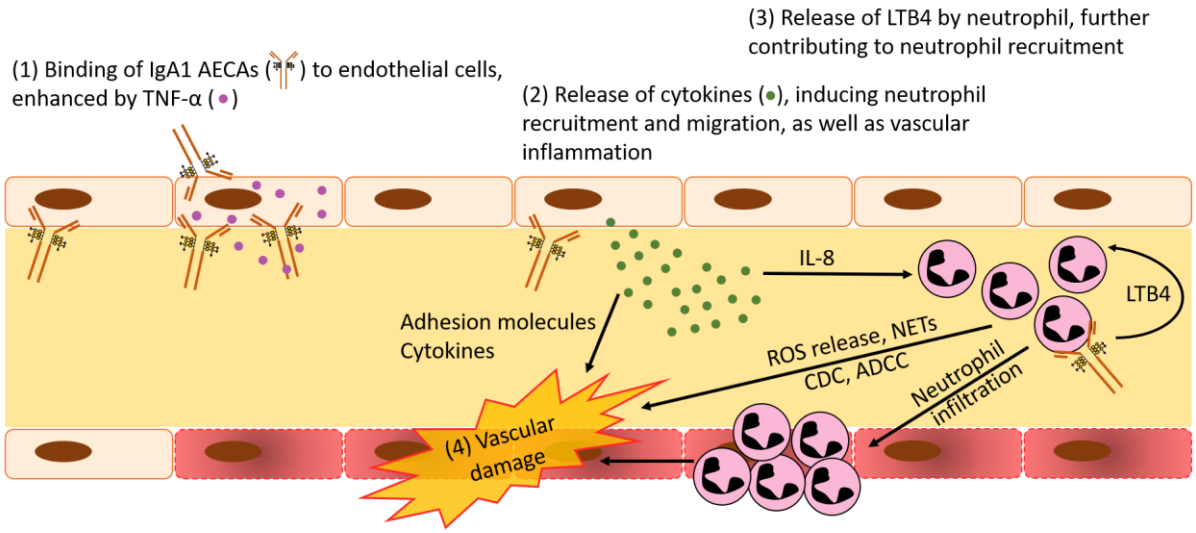


***Figure 1.3**: The potential role of AECAs in IgAV, adapted from Heineke et al. (45). LTB4: leukotriene B4; ROS: reactive oxygen species. NETs: neutrophil extracellular traps. CDC: complement mediated cytotoxicity. ADCC: antibody-dependent cell-mediated cytotoxicity.*

1.1.3.5. *The role of the complement system*

The complement system is an important link between the innate and adaptive immunity. It consists of an activation cascade or around 50 proteins, mainly synthesised by the liver, and it

"complements the antibacterial activity of antibodies" (50). It can be activated through three different routes: the classical pathway, the mannose-binding lectin pathway and the alternative pathway. Regardless of the route used, all pathways converge at C3 activation: cleavage of C3 by C3 convertases produces C3a and C3b, which leads to subsequent cleavage of C5 (forming C5a and C5b). During this process, the anaphylatoxins C3a and C5a are produced, further promoting inflammation through cytokine release and recruitment of immune cells. In addition, C3b also leads to opsonization of pathogens by phagocytosis and C5b binds to C6, C7, C8 and C9 to form the membrane attack complex (MAC, C5b-9), leading to cell lysis. The classical pathway is activated by binding of C1q to antigen-antibody complexes (mainly IgG and IgM) while the mannose-binding lectin pathway uses mannose-binding lectins (MBLs) and ficolins to identify carbohydrate patterns found on the surface of pathogens. In contrast to the two aforementioned pathways where complement activation is triggered by the recognition of exogenous materials, the alternative pathway is continuously activated at low level by spontaneous hydrolysis of C3, which ultimately leads to the production of C3BbB and covalent binding of C3b to activating surfaces (i.e., bacterial surfaces). This phenomenon amplifies alternative pathway activation and enhances C3 production which further activates the common pathway. This is regulated by various factors such as factors D, H and I, MCP-1, properdin, or complement receptor 1. The alternative pathway also functions as an amplifier for any of the other two pathways through C3b: the amplification loop is in fact responsible for 80% of terminal pathway activation (51-53). Complement activation is summarised in **Figure 1.4**.

Growing evidence supports the role the complement system plays in IgAV (44, 45). The classical pathway is thought to not be activated in IgAV and IgAVN due to the general absence of C1q deposits, although some degree of classical pathway activation in the skin biopsies of adult patients with IgAV has been reported by one recent study (12% of cases positive for C1q) (54). Activation of both the mannose-binding lectin and alternative pathways have been reported in the skin, kidneys and sera of patients with IgAVN (54-57). Dysregulation of the amplification loop or genes polymorphisms of certain complement factors have also been proposed to play a role in IgAV phenotype (58). In addition, C5b-9 and C4d positivity in renal biopsies was associated with poor renal outcomes in patients with IgAVN and IgA nephropathy (59). Finally, C5a can increase production of IL-8, MCP-1, E-selectin, and ICAM-1 *in vitro* (57) and participates in neutrophil chemotaxis.

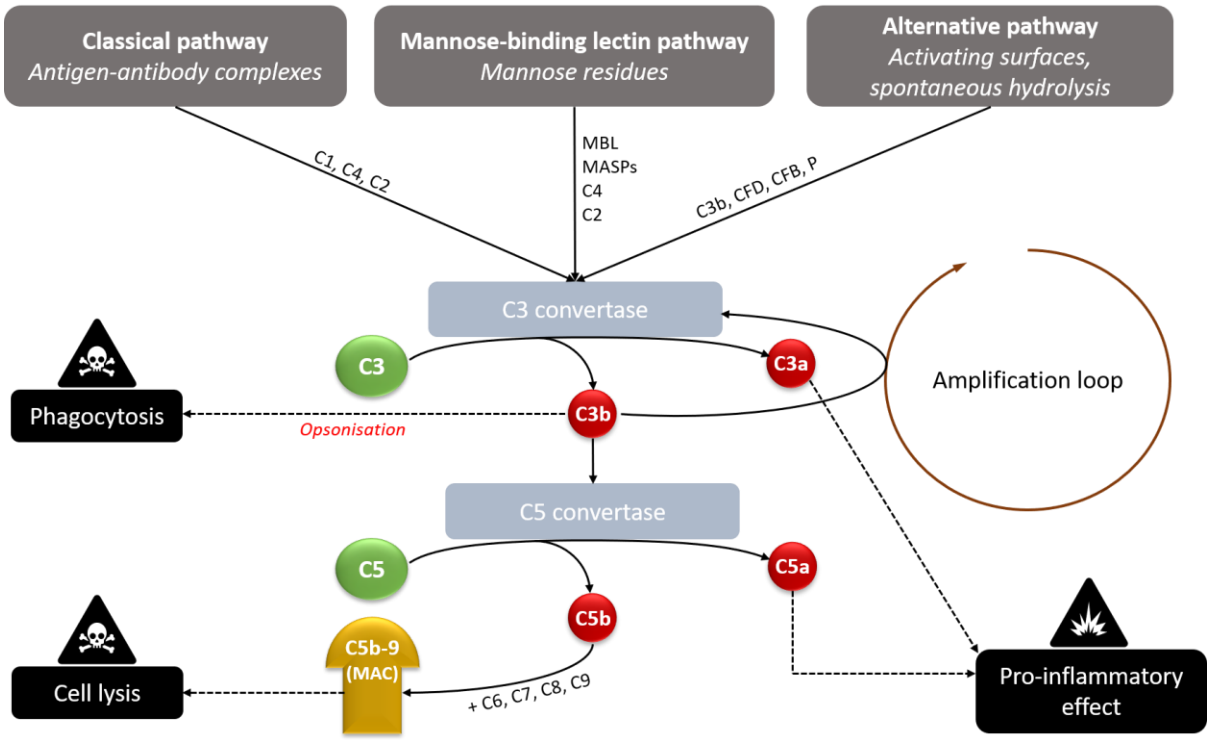


***Figure 1.4**: A simplified summary of the complement system, adapted from Girardi et al (60). MBL: mannose-binding lectin; MASPs: MBL-associated serine proteases; CFD: complement factor D; CFB: complement factor B; P: properdin; MAC: membrane attack complex.*

1.1.3.6. *Inflammatory mediators involved*

Several inflammatory mediators have been identified in patients with IgAV. The main cytokines found in the serum of both adult and children with IgAV was summarised by Sugino and colleagues in 2021 and this is reproduced in **Table 1.1** (47). Generally, proinflammatory cytokines (IL-1β, IL-6, IL-8, IL-17A, TNF- α, IFN-γ) and adaptive immunity cytokine IL-4 serum levels are elevated whereas anti-inflammatory cytokine IL-10 levels are reduced. Interestingly, TNF-α and IL-17 inhibitors may possibly induce IgAV (44, 47), which highlight a complex relationship between these cytokine levels and IgAV pathogenesis.

Cytokine	IgAV	IgAVN
IL-1β	↑ Increased (1)	↑ Increased (1)
	→ Unchanged (2)	→ Unchanged (2)
IL-2	→ Unchanged (1)	→ Unchanged (1)
IL-4	↑ Increased (1)	↑ Increased (1)
IL-6	↑ Increased (4)	↑ Increased (4)
IL-8	↑ Increased (2)	↑ Increased (2)
IL-9	→ Unchanged (1)	-
IL-10	→ Unchanged (2)	→ Unchanged (2)
	↓ Decreased (1)	↓ Decreased (1)
IL-12p70	→ Unchanged (2)	→ Unchanged (2)
	↓ Decreased (1)	↓ Decreased (1)
IL-17A	↑ Increased (1)	↑ Increased (1)
IL-23	→ Unchanged (1)	-
	↓ Decreased (1)	
TNF-α	↑ (2)*	↑ (2)*
	→ Unchanged (2)	→ Unchanged (3)
IFN-γ	↑ Increased (1)	↑ Increased (1)
		→ Unchanged (1)

***Table 1.1**: Cytokine profile in the serum of patients with IgAV, reproduced from Sugino et al. under Creative Common CC BY license (47). *the original table stated decreased TNF-α levels for one study, since then a corrigendum (61) was published by the authors of the primary study (62). The numbers in brackets represent the number of studies reporting this cytokine change as statistically significant.*

In addition, infiltration of inflammatory cells in the vessel walls is seen in IgAV, predominantly neutrophils (44). Neutrophil infiltration results in NETs release and further neutrophil recruitment, leading to tissue damage, as described in section **1.1.3.4**. Cellular immunity is also likely to be involved (44). Elevated serum IL-17 levels and increased number of peripheral Th17 cells in children with IgAV suggest they contribute to vascular inflammation (63). In adults, CXCL10 and CXCL11 blood levels are increased and may participate to the recruitment of CXCR3-expressing T cells in the skin and kidneys, the degree of infiltration was shown to correlate with the severity of kidney damage (64). Finally, cytotoxic T cells and natural killer cells play a key role in the development of nephritis in IgAV (65).

1.1.3.7. *Summary of the pathophysiology of IgAV*

In summary, the pathophysiology of IgAV is complex and not fully understood, hence the need for more research. The exact mechanisms underlying the vasculitis and IgA deposits in the absence of renal involvement are unclear, although a strong auto-immune response is triggered, driven by neutrophils infiltrates, pro-inflammatory cytokines and complement activation. Gd-IgA1-CICs role in

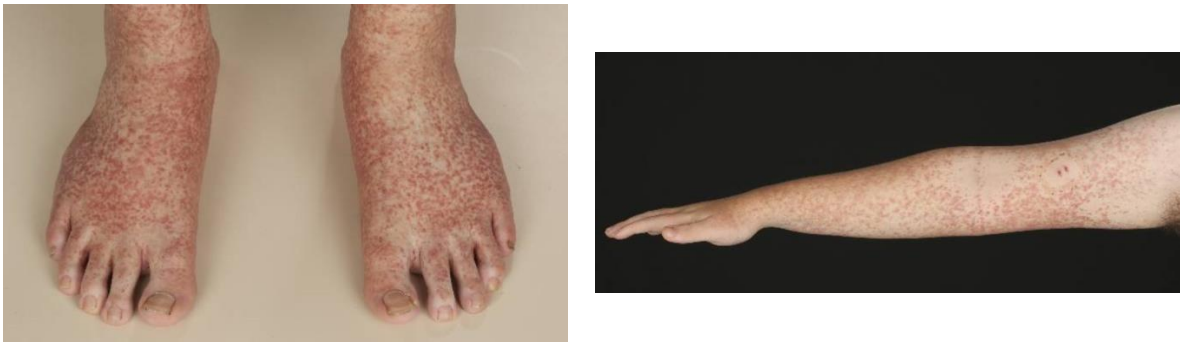
IgAV is controversial whilst it is well recognised they are responsible for mesangial activation in IgAVN, in a similar manner to IgA nephropathy.

1.1.4. Clinical features

IgAV is a multisystemic disease which usually presents acutely in previously well children after a non-specific intercurrent infection (19). The main clinical feature is a palpable purpuric non-blanching skin rash, which can be associated with involvement of the musculoskeletal, gastrointestinal and renal systems. In males, scrotal inflammation can also be seen. Extremely rarely, the inflammation can extend to the respiratory and nervous systems and this can be severe (66).

1.1.4.1. *Cutaneous involvement*

The rash is typically an erythematosus petechial or purpuric rash and tends to follow a symmetrical distribution. It can be found on the whole body but most frequently involves the lower limbs and buttocks, especially the extensor surfaces of the legs and feet (**Figure 1.5.a**). The rash may extend to the upper limbs and the trunk (**Figure 1.5.b**), although it is less common and it is unknown whether a more extensive rash is associated with poorer outcomes. The areas of purpura vary in size, are often palpable with slightly elevated lesions which do not fade with pressure (i.e., non-blanching). It is usually non-painful but it can be described as itchy. The face is very rarely involved, but it can be seen in severe cases. Patients can also present with atypical skin manifestations, such as necrotic lesions and haemorrhagic bullae which are more common in adults than in children (19, 66).



(a) **(b)**

Figure 1.5*: IgAV typical purpuric rash demonstrating petechia and purpura. The rash is most commonly found on the extensor surfaces of the legs and feet, in a symmetrical distribution* ***(a)****. Less commonly, it can involve the arms* ***(b)****. Also note the skin biopsy scar on the arm of this patient who presented with atypical disease. Informed written consent was obtained for the use of these images.*

1.1.4.2. *Musculoskeletal involvement*

Between 70 to 90% of patients will present with musculoskeletal involvement during the acute phase, in the form of arthralgia and/or oligoarthritis (9, 67). The most commonly involved joints are those of the feet, ankles and knees (19). In up to 25% of patients, arthritis was reported to precede

purpura onset (68). Joint manifestations of IgAV are usually short-lived and are not known to cause any long-term joint damage (19).

1.1.4.3. *Gastrointestinal involvement*

Gastrointestinal (GI) symptoms can affect up to 75% of patients (69, 70) and they can occur prior to the onset of the rash, which can lead to misdiagnosis until the rash appears (71, 72). It presents with colicky abdominal pain and is sometimes accompanied by vomiting, GI bleeding (in the form of melaena or haematemesis) and/or diarrhoea (66, 69). Severe complications can arise from GI-involvement secondary to IgAV, these include intussusception, perforation, obstruction, as well as haemorrhage (19, 66).

1.1.4.4. *Renal involvement*

In IgAV, renal involvement, termed IgAV nephritis (IgAVN) is the main contributor to long term morbidity and mortality. It can present with a spectrum of manifestations which include microscopic haematuria/proteinuria, nephritic/nephrotic syndrome (7, 19) and rarely rapidly progressive glomerulonephritis (73). Purpura onset is almost never preceded by nephritis. Macroscopic haematuria can be seen in a small number of patients, it tends to be transient in the acute phase but some patients can develop recurrent macroscopic haematuria which is similar to that seen in IgA nephropathy. Nephritic, nephrotic or a mixed nephritic/nephrotic syndrome can occur and require specialised tertiary management (74). Although the renal involvement can affect 40-50% of patients, it is usually asymptomatic and self-limiting in the majority of cases (19). Risk factors for the development of IgAVN include age > 10 years old at presentation, male gender, severe gastrointestinal symptoms, arthritis/arthralgia, persistent or recurrent IgAV, white blood cells (WBC) > 15 × 109/L, platelets > 500 × 109/L, elevated antistreptolysin O (ASO), and decreased C3 (7).

1.1.4.5. *Other*

In male patients, inflammation of the genitalia (most commonly epididymitis or orchiditis) can be seen and presents as acute scrotal swelling mimicking testicular torsion (75). Early assessment is important to exclude testicular torsion, which is not caused by IgAV (19). Scrotal involvement secondary to IgAV was reported to affect 2 to 38% of male children and is reported to be responsible for 3% of all cases of acute scrotal presentations (75, 76).

Lung involvement is very rare and tends to be more common in adult patients. It can manifest as diffuse alveolar haemorrhage which can be life-threatening (77). Even rarer, the nervous system can be involved, usually in young children with a severe disease course. Central nervous system manifestations include IgAV-related encephalopathy, cerebral vasculitis, cerebral haemorrhage, posterior reversible encephalopathy syndrome and seizures (66). Although the incidence of IgAV

manifestations in such uncommon sites is extremely low, they result in a poor prognosis and it can be challenging for clinicians to differentiate these IgAV complications from other diseases.

1.1.5. Diagnosis and classification

In 2019 the first European consensus-based recommendations for the diagnosis and management of IgAV by the SHARE initiative (Single Hub and Access point for paediatric Rheumatology in Europe) were published (78). They recommended the use of the EULAR/PRINTO/PReS Ankara 2008 criteria as a diagnosis tool for children with IgAV, which is summarised in **Table 1.2**. The presence of purpura or petechiae with lower limb predominance is required with either abdominal pain, arthritis/arthralgia, renal involvement and/or histology demonstrating leukocytoclastic vasculitis with predominant IgA deposits (79). In children, it reaches excellent sensitivity and specificity with 100% and 87% respectively whereas in adults, the American College of Rheumatology (ACR) criteria has been traditionally used (80) but lacks specificity, sensitivity and is now outdated. Plus, the EULAR/PRINTO/PReS criteria were found to outperform the former in an adult cohort (81).

Criterion	Definition
Purpura (*mandatory*)	Purpura (commonly palpable and in crops) or petechiae, with lower limb predominance, in the absence of thrombocytopenia.
AND either one of the following	
Abdominal pain	Diffuse colicky abdominal pain with acute onset. May include intussusception and gastrointestinal bleeding.
Histology	Histology demonstrating leukocytoclastic vasculitis with predominant IgA deposits or proliferative glomerulonephritis with predominant IgA deposits.
Joint involvement	Arthritis of acute onset: joint swelling or joint pain with limitation on motion. Arthralgia: joint pain without joint swelling or limitation on motion.
Renal involvement	Proteinuria >0.3 g/24 h or >30 mmol/mg of urine albumin/creatinine ratio on a spot morning sample. Haematuria or red blood cell casts: >5 red blood cells/high power field or red blood cells casts in the urinary sediment or ≥2+ on dipstick.

***Table 1.2**: The EULAR/PRINTO/PReS classification criteria for childhood IgAV. Reproduced from Ozen et al. (79).*

1.1.5.1. *Renal involvement classification*

Renal involvement was defined as either proteinuria and/or haematuria by the EULAR/PRINTO/PReS criteria (**Table 1.2**) (79). The SHARE initiative further classified the severity of renal involvement, based on urinary findings and/or renal histology, which is presented in **Table 1.3**.

IgAVN severity	Definition
Mild	Normal GFR (>80 mL/min/1.73m2) and mild (UP:UC ratio <100 mg/mmol) or moderate (UP:UC ratio 100–250 mg/mmol) proteinuria.
Moderate	<50% crescents on renal biopsy and impaired GFR (<80 ml/min/1.73 m2) or severe persistent proteinuria*.
Severe	>50% crescents on renal biopsy and impaired GFR (<80 ml/min/1.73 m2) or severe persistent proteinuria*.
Persistent proteinuria	UP:UC ratio >250 mg/mmol for 4 weeks. (*severe persistent proteinuria) UP:UC ratio >100 mg/mmol for 3 months. UP:UC ratio >50 mg/mmol for 6 months.

***Table 1.3**: Severity of renal involvement in IgAVN, adapted from Ozen and colleagues (78). UP:UC ratio: urinary protein to urinary creatinine ratio. GFR: glomerular filtration rate.*

1.1.5.2. *Histological diagnosis*

1.1.5.2.1. Renal biopsy

Performing a kidney biopsy remains the gold standard to confirm the diagnosis and assess the degree of renal inflammation in IgAV, however, it remains a highly invasive procedure and carries risks. A group of nephrologists issued recommendations for performing a kidney biopsy in children with IgAV, based on the recommendations of the SHARE initiative and Kidney Disease: Improving Global Outcomes (KDIGO) alongside suggested terminology improvements from an international workshop. Persisting severe/moderate proteinuria (see **Table 1.3**), acute kidney injury stage 1 or greater and nephrotic syndrome were proposed to be absolute indications for conducting a renal biopsy (82).

In IgAVN, immunofluorescence demonstrates predominant IgA1 deposits in the mesangium alongside glomerular deposits of IgG, IgM and C3. Mesangial hypercellularity with increased mesangial matrix, endo-capillary hypercellularity, segmental glomerular sclerosis, cellular crescents, tubular atrophy and/or interstitial fibrosis can be seen on light microscopy (19). Histological classification is based on the International Study of Kidney Disease in Children (ISKDC) classification for IgAV nephritis (**Table 1.4**), which was initially proposed in 1977 (83). The ISKDC has been recently criticised for focusing almost exclusively on glomerular crescents and for its lack of accuracy (23). Other systems have been proposed to succeed the ISKDC, such as modified semiquantitative classification (SQC) scores (84) or the Oxford classification (MEST-C score) (85), which is widely accepted for IgA nephropathy but still under validation for its role in IgAVN (86).

ISKDC Grade	Features
Grade I	Minimal changes, normal light microscopy
Grade II	Mesangial proliferation
Grade III	Crescents < 50% of the glomeruli; A: Focal, B: Diffuse
Grade IV	Crescents 50–75% of the glomeruli; A: Focal, B: Diffuse
Grade V	Crescents > 75% of the glomeruli
Grade VI	Membranoproliferative glomerulonephritis

***Table 1.4**: The grading system of the International Study of Kidney Disease in Children (ISKDC) classification for pathological scoring of IgAVN in children (83).*

1.1.5.2.2. Skin biopsy

Performing a skin biopsy is only recommended in children with an atypical rash, defined as extensive lesions or diffusely distributed lesions. In addition, the absence of IgA staining on a skin biopsy does not exclude a diagnosis of IgAV. Skin biopsies can be useful to exclude alternative diagnoses, such as antineutrophil cytoplasmic antibodies (ANCA)-associated vasculitis (78).

1.1.6. Disease monitoring – current practice

1.1.6.1. *Initial assessment*

The initial assessment of children presenting with IgAV mainly consists of a comprehensive history and clinical examination, as IgAV is diagnosed based on clinical features. First line investigations should be blood pressure (BP) measurement, early morning urinalysis (to identify any proteinuria / haematuria suggestive of renal involvement) and assessment of renal function through creatinine and electrolytes. A full blood count, a coagulation panel and/or a septic screen may be considered to exclude other causes, such as idiopathic thrombocytopenic purpura or sepsis (particularly meningitis), in line with the clinical history. Elevated serum IgA is common but it is not specific to IgAV. Other autoimmune tests (i.e., ANCA autoantibodies, antinuclear autoantibodies, complement levels) may help to differentiate IgAV from other vasculitides in more complex cases, although these are rarely needed or ordered at first presentation. An abdominal ultrasound may be warranted in cases with severe abdominal pain, to exclude intussusception or intestinal perforation. Similarly, an ultrasound of the testes may be conducted to evaluate scrotal pain and swelling (37).

1.1.6.2. *Renal follow up*

IgAVN is the most recognised long-term complication arising from IgAV. As a result, consensus is that all patients should have renal monitoring for at least 6 months following the acute episode, even with a normal urine dipstick at presentation. A normal urine dipstick at presentation is reassuring and the absence of proteinuria at onset was reported to be a good prognostic marker. However, there is no universally agreed follow up algorithms and they vary across centres (74, 87). The Alder Hey

Henoch Schönlein purpura nurse-led pathway was published in 2012 and provides a framework for renal follow up in childhood IgAV that many centres have adopted. Renal monitoring mainly consists of a series of BP measurements and urinalysis to identify any worsening proteinuria/haematuria, which would suggest evolving nephritis. In the Alder Hey pathway, patients with normal urinary findings at presentation follow the "standard" pathway with reviews at day 7, 1 month, 3 months and 6 months whereas patients with urinary abnormalities at presentation or during follow up are reviewed more frequently (87). Criteria to refer patients to paediatric nephrology are presented in **Table 1.5**. Nonetheless, proteinuria lacks sensitivity, it tends to be a later marker of kidney inflammation and its exact value does not correlate with the renal prognosis (87).

Referral criteria
• Hypertension (BP ≥ 95th centile for height) on 3 separate readings
• UACR > 200 mg/mmol
• UACR 100-200 mg/mmol and increasing trend
• Macroscopic haematuria for > 7 days
• Serum albumin < 35 g/dL
• Reduced eGFR (< 90 mL/min/m^2)

Table 1.5: *Criteria to refer children with IgAV to paediatric nephrology, from the Alder Hey HSP nurse-led pathway (87). BP: blood pressure; UACR: urinary albumin to creatinine ratio; eGFR: estimated glomerular filtration rate.*

1.1.7. Outcome

The disease course is self-limiting and symptoms self-resolve in the first month for the vast majority of children. The outcome is excellent with 94% achieving full spontaneous recovery within 2 years (88). IgAV complications can be divided into short-term (usually due to GI-related involvement), medium term (recurring or persisting disease) and long-term complications (mainly related to renal involvement).

1.1.7.1. *Short-term complications*

Acute complications are mainly caused by GI involvement, which is the main reason for hospitalisation in children with IgAV (69). It is usually due to severe abdominal pain, intussusception or GI bleeding and immunosuppression may be warranted. Acute joint pain/swelling can occur with sometimes children refusing to weight bear, which may require overnight admission for pain relief. Although it is very rare, involvement of uncommon sites such as the lungs or the nervous system can be fatal (66).

1.1.7.2. *Medium-term complications – recurring and persisting disease*

Despite its self-limiting course, it is estimated that on average a third of children with IgAV will relapse (89), although recurrence rates greatly vary in the literature, these have been reported to

range from 2.6% to 66% (9, 12, 14, 90-93). Most of the time, recurrences tend to mimic the first episode and involve the reappearance of the skin lesions associated with abdominal pain, joint pain and/or renal involvement (14, 94, 95), although no formal definition exists. Recurrence typically occurs in the first 6 months following the acute phase but subsequent episodes are usually milder and shorter than the first episode (9, 14, 93-95). Late recurrent episodes, months or even years after the initial presentation, are rare but they do occur (9, 93). In addition, the incidence of renal involvement with recurrent IgAV has been reported to be 2.7-11 fold that of patients without relapsing disease in a meta-analysis (7). However, the association between recurrence and poorer renal outcomes remains unclear (11).

Persisting disease, mainly occurs in the form of a persisting rash (persisting purpura) and/or persisting haematuria/proteinuria. Persisting IgAV in children has been reported in several cohort studies but remains poorly defined and is not well documented in the literature (12, 95-99).

1.1.7.3. *Long-term complications*

Renal involvement secondary to IgAV is the main contributor to long term complications and poor prognosis. IgAVN can affect 40-50% of children but it is mainly self-limiting and the majority of children will fully recover (19). However, it is estimated that 1-2% of children with IgAV will develop CKD 5 and will require renal replacement therapy in the form of dialysis or kidney transplant (100). Renal damage induced by IgAVN is usually slow progressing and may only cause problems decades after the first episode. In children with biopsy-proven IgAVN, 20% will have significant proteinuria after 10 years (101). After 20 years following the initial presentation, up to 21% were reported to develop CKD 5 whilst 35% had severe renal impairment (102, 103). In addition, Ronkainen and colleagues found that 70% of pregnancies were complicated by hypertension and/or proteinuria in females who had IgAVN as a child (102). Surprisingly, the incidence of renal complications related to IgAVN has not decreased in the last 50 years despite recent advances in medical research (19, 104). Risk factors of a poor renal outcome in children with IgAVN include older age at onset, lower GFR, nephrotic/nephritic syndrome features at onset and a renal biopsy demonstrating crescentic nephritis (ISKDC grades III-V) (11).

Apart from nephritis, children with IgAV may be at increased risk of developing functional gastrointestinal disorders, such as irritable bowel syndrome or functional abdominal pain syndrome although limited evidence exists in this area (105).

1.1.8. Management

There is no standardised treatment algorithm for IgAV. However, evidence-based recommendations for the treatment of IgAV in children have been issued in 2019 as a result of a

European consortium initiative (the SHARE initiative) (78). These recommendations were mainly based on experts' recommendations due to the lack of clinical trials in this disease, which can be hard to justify due to its self-limiting course.

In the vast majority of patients, treatment is supportive and consists of simple analgesia (i.e., paracetamol and non-steroidal anti-inflammatory drugs -NSAIDs-) to manage joint pain and/or abdominal pain. NSAIDs are not contraindicated in the absence of renal involvement or in the presence of microscopic haematuria without proteinuria and with normal renal function. Corticosteroids (CS) are indicated in orchiditis, cerebral vasculitis, pulmonary haemorrhage and other severe or life-threatening vasculitis manifestations. They may also be considered for severe abdominal pain and rectal bleeding. Oral prednisolone is recommended as first line therapy and intravenous (IV) pulsed methylprednisolone may be used in severe organ involvement (78).

The SHARE guideline did not include any recommendations for children presenting with recurring or relapsing disease without any significant renal involvement, who may warrant disease modifying agents such as immunosuppression (19). Mycophenolate mofetil, azathioprine, rituximab and methotrexate may have a role in managing these more complex cases, although the evidence is sparse and based on small studies and individual case reports (106-111). Skin involvement does not usually require any treatment but refractory cases may benefit from oral corticosteroids, colchicine, dapsone or azathioprine, as reported by small case series (111-116).

1.1.8.1. *Management of IgAVN*

Management of IgAVN in children is currently based on the SHARE recommendations, as further supported by the 2021 KDIGO glomerulonephritis guideline (117). The treatment algorithm proposed by the European consortium is presented in **Table 1.6**. Briefly, oral prednisolone should be used as first line treatment and add-on immunosuppressant therapy can be considered depending on the nephritis severity. RAAS inhibition, in the form of an ACE inhibitor or an angiotensin II receptor blocker (ARB) should be initiated in patients with persistent proteinuria (> 3 months) to prevent or limit glomerular injury (78). No evidence supports the use of corticosteroids to prevent nephritis in children with IgAV (118), hence early prophylactic use of CS should not be considered. The working group further highlighted the lack of evidence and the urgent need for more clinical trials investigating treatment options for paediatric IgAVN (78).

Mild nephritis	
1st line	Oral prednisolone
2nd line	Azathioprine
	Mycophenolate mofetil
	IV pulsed methylprednisolone
Moderate nephritis	
1st line	Oral prednisolone AND/OR
	IV pulse methylprednisolone
2nd line	Azathioprine
	Mycophenolate mofetil
	IV cyclophosphamide
Severe nephritis	
1st line	IV cyclophosphamide AND
	Pulsed IV methylprednisolone AND
	Oral prednisolone
2nd line	Azathioprine OR mycophenolate mofetil AND
	Steroid therapy

***Table 1.6**: Treatment algorithm for IgAVN in children based on nephritis severity, as proposed by Ozen et al (78). For IgAVN severity definitions, refer to **Table 1.2**.*

1.1.9. The overlap between IgA vasculitis and IgA nephropathy

IgA nephropathy (IgAN) was first described by French nephrologists Berger and Hinglais in 1968 when Berger reported IgA deposits similar to what was described in Henoch Schönlein purpura nephritis (119). Since then, many reports described family clustering of both IgAN and IgAV as well as progression from IgAV to IgAN (or vice versa, although rarer) in the same group of patients (23, 120).

On the one hand, IgAV can occur at any age but it is more common in young children whereas IgAN is seen more frequently in young adults. IgAV is diagnosed as a purpuric non-blanching rash associated with either abdominal pain, joint involvement and/or nephritis whilst IgAN is solely confined to the kidneys (120). IgAN most commonly presents as macroscopic haematuria which is not as common in IgAVN, but it can be seen (19, 74).

On the other hand, URTIs are well-recognised triggers for both IgAV and IgAN and histologically, it is often impossible to distinguish between glomerulonephritis caused by IgAN or IgAVN. In addition, IgAN and IgAVN are thought to share the same pathophysiological features: mainly mesangial deposition of gd-IgA1 containing immune complexes, neutrophil infiltrates and, more recently, suggestions of overactivation of the complement system (120). The disease course is more severe in adults for both diseases, and whether or not outcomes are worst for IgAN or IgAVN remains controversial (120).

More clinical studies comparing adults and children with IgAN and IgAVN are required to confirm any similarities or differences and their implications in terms of diagnosis, disease monitoring, prognosis and treatment (120).

1.2. The IgA Vasculitis study

The core work of this thesis results from the IgA Vasculitis study (IgAV study), which is a prospective observational longitudinal established in 2019 at Alder Hey Children's NHS Foundation Trust, Liverpool, UK The below is a summary of the IgAV study protocol version 1.3 (17/12/2020) (*unpublished*) (121).

1.2.1. Study aim and objectives

The overall aim of the IgAV study is to conduct research through the development of a clinical cohort of children with IgAV and corresponding biological samples in order to improve the care and scientific understanding of IgAV, with a focus on reducing the incidence of chronic kidney disease. The key objectives of this study were to understand the biological mechanisms and clinical features of the disease.

1.2.2. Study design

The IgAV study is a single-centre prospective observational longitudinal cohort study using participants attending Alder Hey Children's NHS Foundation Trust, Liverpool (UK) for clinical data collection and serial sampling.

1.2.2.1. *Inclusion criteria*

The inclusion criteria for each group are presented in **Table 1.7.** For the purpose of this work, only the IgAV and healthy controls (HCs) cohorts were used.

Group	**n**	**Inclusion criteria**
Patients with IgAV	200	< 18 years old with a clinical diagnosis of IgAV according to the EULAR/PRINTO/PReS criteria (79)
Disease controls	150	
Inflammatory controls	50	< 18 years old with systemic lupus erythematosus (SLE) fulfilling 4 of the revised SLE ACR criteria (122)
IgA controls	50	< 18 years with biopsy proven IgA nephropathy
Dental controls	50	< 18 years without IgAV attending for dental interventions
Healthy controls	50	< 18 years otherwise healthy attending for non-inflammatory day case investigations or surgery with not known past medical history and not taking any medication (i.e., attending for growth assessment, nevus excision, laser therapy, etc)

Table 1.7*: Inclusion criteria to the IgAV study as per the study protocol (121).*

1.2.2.2. *Exclusion criteria*

The exclusion criteria were as follow: *(i)* no diagnosis of IgAV (apart for the healthy and disease controls); *(ii)* diagnosis uncertain or in doubt; *(iii)* patients unable or unwilling to consent.

1.2.3. Identification of patients and recruitment

The identification of suitable patients was performed through weekly screening of the medical day case unit (MDU) list by searching for "HSP" appointments as well as by exploring the clinic list of the lead renal consultant and principal investigator for this study (LO) on Meditech version 6.0 for Windows (Medical Information Technology Inc, MA, USA). Patients were approached mainly in MDU during their follow up appointments as per the Alder-Hey nurse led-HSP pathway or in the renal clinics. Occasionally, patients could be recruited on the wards or in theatre if they were an acute inpatient or scheduled to have a kidney biopsy.

HCs were recruited in theatre/allergy clinics/endocrine clinics to provide age- and sex-matched samples and the identification of suitable candidates was performed through screening of the corresponding lists.

Clinicians, physician associates, research nurses and research students were involved in the identification and recruitment of participants to the study.

As of June 26th, 2022, the study had recruited 88 patients with IgAV and 32 HCs. The recruitment of the disease control subgroups has not commenced yet to allow staff to focus on building the core biorepository. The COVID-19 pandemic had a dramatic impact on research studies in general, including the IgAV study which was forced to pause during the first lockdown. Recruitment resumed after the relaxation of restrictions following the first wave of the pandemic, and it has been steadily increased ever since. A summary of the recruitment numbers since the opening of the study in 2019 is presented in **Figure 1.6**.

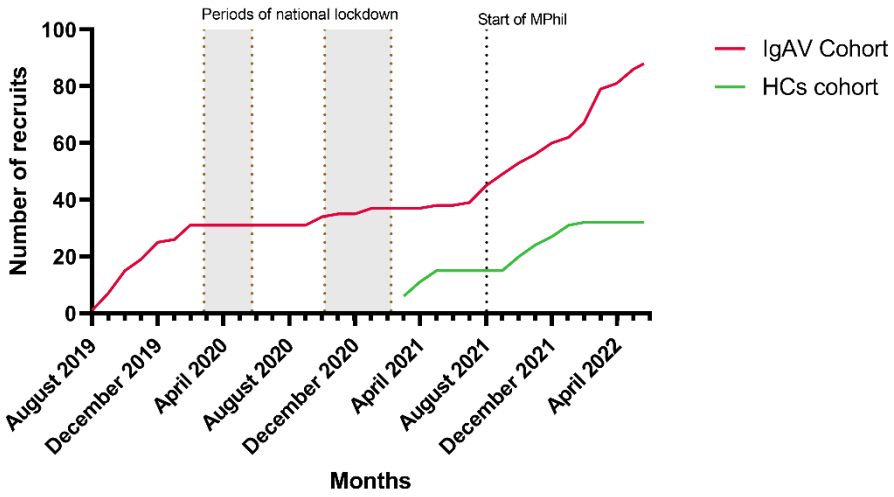


***Figure 1.6**: Number of recruits to the IgAV Study over time since the opening of the study. The periods of national lockdown are highlighted in grey.*

1.2.4. Sample collection and processing

The biosamples were obtained at recruitment and each clinical encounter. These could include urine (up to 10 mL), saliva (up to 10 mL) and blood (plasma 2mL; serum 2mL) samples. Blood and saliva samples were taken only if samples were being obtained for clinical purposes, i.e. at the time of other clinical investigations, during anaesthetic or cannula insertion (especially for the HCs). Biological samples were processed in accordance with a standard operating procedure. Briefly, healthy control urine samples were tested for bacterial contamination using urine dipstick testing and they were discarded if they demonstrated positivity for leukocytes, nitrites, blood or > +1 for protein. Urine and serum samples were centrifuged at 300×g for 10 mins, transferred into a new falcon tube, centrifuged again at 300×g for 10 mins and aliquoted into 1mL (urine) and 200µL (serum, plasma) sterile Eppendorf tubes for storage. Samples were stored at -80 °C in the Institute in the Park building (Alder Hey Children's NHS Foundation Trust, Liverpool, UK) to constitute the IgAV study biorepository.

1.2.5. Data collection

Data collection was performed on hard paper copies using case report forms (CRF). Clinical data collection included the following:

- Routine demographic data
- Any family history or trigger events
- Diagnostic criteria
- Date of symptoms onset
- Date of diagnosis
- Renal monitoring
- Blood results
- Skin or renal biopsy results
- Additional immunological investigations
- Treatment used, indication, doses and duration
- Outcome at 12 months post diagnosis
- Child oral health score from questionnaire

This year was the first time the clinical data were used to correlate with the laboratory experiments, and this highlighted the need for areas of improvement. Following feedback from the multi-professional team, an updated version of the CRF was developed (**Appendix 2**).

1.2.6. Regulatory approvals and consent

All procedures involving human subjects were conducted in accordance with NIHR Good Clinical Practice, HTA Codes of Practice, the Declaration of Helsinki and comparable ethical standards. The IgA Vasculitis study was approved by HRA and Health and Care Research Wales (HCRW) on June 21st, 2019 (REC 17/NE/0390, protocol UoL001347, IRAS 236599) to run for a duration of 5 years (until December 17th, 2025). The study received local regulatory approvals to begin recruitment in August 2019. Written informed consent was obtained from parents and children (>16) prior to any study-related procedure. In children able to understand the process but unable to consent, written assent was obtained in addition to parental consent. Regardless of their age, children were included as much as possible in the recruitment process and received a certificate of thanks.

1.3. Research gaps in understanding disease activity in children with IgAV

Disease activity is generally defined as the "aspects of a patient's disease that are potentially reversible". Disease damage, on the other hand, refers to "the irreversible manifestations of the disease or its treatment". Both disease activity and damage ultimately contribute to disease severity, although disease activity and severity are often used interchangeably. In rheumatological diseases, a disease activity measure is an attempt to quantify the inflammatory process of the disease. Such measures can include biochemical markers, quantification of the amount of inflamed tissue, or clinical and psychological consequences of the disease process. A combination of these is often use to create scoring tools in order to objectively assess and monitor disease activity, taking into account the multidimensional aspect of paediatric rheumatic diseases (123).

In paediatric vasculitides, the Paediatric Vasculitis Activity Score (PVAS) is considered to be the gold standard for measuring disease activity. It contains 64 features of various active vasculitides, each referring to one of nine organ-based systems, and it was developed and adapted from its adult version, the Birmingham Vasculitis Activity Score (BVAS) in 2012 (124, 125). However, this tool is not validated for IgAV. As a result, children presenting with an atypical disease course represent a clinical and therapeutic challenge to clinicians due to the lack of standardised definition and high-quality evidence on how best to manage these cases. Although this occurs in the minority of children with IgAV, better describing this group of patients alongside standardising definitions for recurrent and persisting disease would help develop clinical guidelines and core outcomes as a foundation for future clinical trials.

Similarly, the incidence of renal complications in IgAV has not decreased over the last few decades, despite advances in medical research (19, 104). Half of the children presenting with IgAV can have some degree of nephritis, and although the majority will have an excellent outcome, renal sequelae remains a concern as the incidence of CKD 5 in the long term is still significant (102, 103). Performing a renal biopsy is the gold standard for obtaining the diagnosis, however it remains invasive and only provides transient information with limited ability to provide an accurate assessment of disease activity (126).

Current clinical practice is that all children should undergo a period of 6 months of renal monitoring which mainly relies on the degree of proteinuria (19). Proteinuria has limitations as it tends to be a late marker of kidney damage and it is often difficult to discriminate proteinuria from the acute and chronic phases of the disease (126). Currently, there is no way to identify which patients are at high risk of developing nephritis and this period of monitoring following initial presentation offers a "window of opportunity" that could be used for early intervention.

Hence, there is a need for improved understanding and consideration of non-invasive biomarkers offering real-time disease activity evaluation. Biochemical markers are important tools for the diagnosis, treatment and monitoring of disease progression. They are often used in combination with other diagnostic tools (127). A systematic review of the literature recently identified urinary biomarkers of in children with IgAVN, of which kidney injury molecule-1 (KIM-1), monocyte chemotactic protein-1 (MCP-1), N-acetyl-β-glucosaminidase (NAG) and urinary angiotensinogen (UAGT) were the most promising in identifying the presence and/or severity of IgAVN (128). Urine offers an ideal biofluid for the analysis of nephritis to monitor disease progression and to uncover potential biomarkers which may be more indicative of IgAVN. Urine is also readily available in large volumes and is non-invasive. Identifying urinary proteins which are indicative of nephritis in children with IgAVN may help elucidate mechanisms of disease, identify potential new treatment targets and give directions for future research.

1.4. Aim of the thesis

The overall aim of this thesis was to improve the understanding of disease activity in children with IgA vasculitis. First, this thesis focused on describing a cohort of children with recurrent/persisting disease to identify how their disease was monitored alongside potential ways of standardising definitions in order to improve their care. Then, this work aimed to identify differences in the urinary concentrations of IgA and complement C5a in the urine of children with IgAV and any relationship with nephritis. Finally, this thesis sought to uncover the differences in the urinary proteome between

patients with and without nephritis to provide novel insight into the pathophysiology of IgAVN, identify some potential therapeutic targets and provide directions for future research.

2. A case series on recurrent and persisting IgA vasculitis and a systematic review of the literature

2.1. Introduction

IgA vasculitis is usually self-limiting and carries an excellent prognosis in most children, with 94% achieving full spontaneous recovery within 2 years (88). Symptoms typically self-resolve within the first 4 weeks (129) but it is estimated that a third of children will relapse, often within 6 months of disease onset (89, 93, 95). Relapses have been described to mimic the first episode and to be not necessarily as severe, however a small proportion of children may have complications arising from a prolonged disease course (93-95). Several risks factors, such as older age at onset or a more severe form at presentation, have been suggested to predispose patients to relapsing IgAV, but reports are inconsistent (9, 92-94, 130). In addition, renal involvement is much more common in patients with recurrent IgAV; however, it does not necessarily correlate to poorer renal outcomes (7, 11). Although children with an atypical disease course have been described in cohort studies, this aspect of the disease remains poorly understood and there is no agreed consensus definition for either recurring or persisting disease nor agreement on its management. Standardisation of care, through disease definitions and clinical guidelines, are important in order to provide equity of care in addition to their role as a foundation for research.

2.1.1. Aims

The primary aim of this chapter was to describe the clinical characteristics of a cohort of children with recurring or persisting IgAV and to identify any factors associated with their disease course. The secondary aim of this chapter was to systematically review the literature to identify potential standardised definitions for recurrent or persisting IgAV.

2.2. Materials and methods

2.2.1. Cohort

Patients were selected from a previously identified retrospective cohort that was used to validate the IgA Vasculitis disease Activity Score (IgA-VAS). For this validation study, children (< 18 years of age at presentation) who attended Alder Hey Children's NHS Foundation Trust (Liverpool, UK) between January 1st, 2015 to December 31st, 2019 with a diagnosis of IgAV were included. Patients were identified by the information technology (IT) team using International Classification of Diseases (ICD-10) code D69.0 ("allergic purpura or Henoch Schönlein purpura"). Exclusion criteria were as follows: (i) ≥ 18 years old at presentation; (ii) diagnosis of IgAV uncertain or in doubt; (iii) patients with insufficient available data to score (131).

From this cohort, patients with a history of multiple attendances to our centre outside of the HSP nurse-led pathway (87) were first identified by a previous student (CW) and their supervisor (LO). This list was further explored to identify any patients diagnosed with recurrent or persisting IgAV.

2.2.2. Definitions

IgAV had to be diagnosed according to the EULAR/PRINTO/PReS 2008 Ankara-endorsed criteria (79). Recurrent disease, persisting disease and remission were defined if they had been stated in a clinical letter by a paediatric rheumatologist or nephrologist (ST3 – specialist registrar- level or above). Renal involvement (IgAV nephritis – IgAVN) was defined as a urinary albumin to creatinine ratio (UACR) > 30 mg/mmol at any time during follow up (78, 79). From the clinical letters, the main reasons prompting referral were identified as the main presenting complaint(s), and accessory symptoms not being considered the main reasons for re-presentation were classified as secondary. Severe cutaneous involvement was defined as a typical IgAV rash that was also accompanied by necrotic, bullous and/or ulcerative lesions. The age-specific laboratory reference ranges applied at Alder Hey Children's NHS Foundation Trust (Liverpool, UK) were used to identify out of range blood tests (132, 133).

2.2.3. Data collection

Clinical data were retrospectively collected from the clinical notes. Follow up was from diagnosis of IgAV until either discharged or the time of data collection (November 21st, 2021), whichever occurred first. The history, clinical features, treatment received and disease progression were recorded at presentation and at each clinical encounter for which data were available. Any triggers for the relapses were recorded from the letters if documented. In addition, the lowest and highest values of serum C3, C4, IgA, erythrocyte sedimentation rate (ESR) alongside any antinuclear antibodies (ANA) or antineutrophil cytoplasmic antibodies (ANCA) positivity at any time during the follow up were recorded. Any histology score was recorded. The disease burden was evaluated based on what was subjectively reported by the clinicians in the letters.

2.2.4. Handling missing data

It was very likely data would be missing due to the retrospective nature of this study. Due to some children not presenting initially to our centre, we were unable to record baseline blood results and the baseline clinical characteristics were collected from the referral letters or first clinic letters. Patients with no prescribed drugs on the electronic system with notes referring to "simple analgesia" or "occasional analgesia" were considered to be taking paracetamol solely. A rash described as "typical of IgAV" at presentation was considered to be a non-severe rash following a typical lower-limb distribution.

2.2.5. Systematic literature review

A systematic literature review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidance (134) and the Cochrane Handbook for Systematic Reviews of Interventions (135).

2.2.5.1. *Search strategy and eligibility*

The search strategy was adapted using the "Best practice guideline for the management of complications associated with IgA vasculitis (HSP) in children and young people search strategy" (*unpublished*) (136). This review formed part of guideline 2 of the UK IgAV guideline development group (GDG) project (referred to as "the guideline" below), a national, multi-professional collaborative project aiming to develop the first national guidelines for the diagnosis and management of IgAV in children in the UK. This guideline project was endorsed by the Royal College of Paediatrics and Child Health (RCPCH) and UK kidney association following the methods set out for National Institute for Health and Care Excellence (NICE) accreditation.

PubMed, Scopus and Cochrane Central Register of Controlled Trials (CENTRAL) databases were searched for articles published between February 2002 up to February 2022 using a predefined search strategy, which is presented in **Table 2.1**.

Questions from the guideline search strategy	
"1) In children and young people with IgAV under the age of 18 years, what clinical signs or symptoms would support a diagnosis of persisting disease? 2) In children and young people with IgAV under the age of 18 years, what clinical signs or symptoms would support a diagnosis of recurrent disease?"	
Key concepts used for the search	
Concept 1	"pediatric" OR "paediatric" OR "child*" OR "adolescen*"
Concept 2	"immunoglobulin A vasculitis" OR "IgA vasculitis" OR "IgAV" OR "henoch schonlein purpura" OR "HSP"
Concept 3	"recur*" OR "relaps*" OR "reoccur*" OR "persist*" OR "refract*" OR "chronic*"
Search strategy	Concept 1 AND Concept 2 AND Concept 3

***Table 2.1**: Search strategy and key concepts used for the systematic literature review, adapted from the guideline search strategy (136).*

Primary research studies in English providing a clear definition for recurrent or persisting IgA vasculitis in children (< 18 years of age at presentation) that were accessible in full text through the University of Liverpool were included; the full inclusion and exclusion criteria are presented in **Table**

2.2. Definitions of recurrent or persisting purpura were also included if study participants had a prior diagnosis of IgAV whereas definitions of recurrent or persisting IgAVN solely were discarded.

Criteria	Inclusion	Exclusion
Topic	Studies related to IgA vasculitis.	Studies not related to IgAV.
Population	Children and young people aged < 18 years at first presentation (OR same definition used for both children / adults) with a confirmed diagnosis of IgAV.	Studies solely focusing on adults.
Definition	Clear definition of recurrent / persisting IgA vasculitis. Definitions of recurrent or persisting purpura were also separately included separately.	Definition of recurrent IgAV unclear, incomprehensive or too organ-specific (ie definition of recurrent IgAV nephritis solely).
Research type	Primary research.	Secondary research.
Study type	Randomised controlled trials (RCT) If no RCT available to consider; Cohort studies Case series (> 5 patients) Case control studies Meta-analysis Systematic reviews Long term follow-up studies	Case reports Editorials Comments Annotations Letters not containing data Commentaries Updated systematic reviews by same methodology i.e., Cochrane (most recent version will be included) Non-traditional therapies (i.e., Chinese medicines) and surgical intervention
Availability	Full text available through the University of Liverpool.	Full text non-available, unless recurrent or persisting disease was defined in the abstract.
Language	English.	Non-English.

Table 2.2*: Eligibility criteria for inclusion/exclusion from the systematic review. Adapted from the guideline search strategy (136).*

2.2.5.2. *Screening process*

Removal of duplicates was performed using EndNote software version 9.3.3 (Alfasoft Limited, UK) and the search results were uploaded onto the online platform Rayyan (https://www.rayyan.ai/, accessed April 25th, 2022) (137). The titles and abstracts of the identified articles were first screened by two independent reviewers (JM and LO) to exclude irrelevant studies. This process was blinded to the reviewers within Rayyan. The full-texts of the remaining articles were then assessed for inclusion according to the eligibility criteria by one reviewer (JM). Any disagreement at any stage was resolved

by consensus-based discussion. The reference lists of the included articles as well as reviews of interest flagged during the screening process were searched to identify any additional relevant studies.

2.2.5.3. *Quality assessment and data extraction*

The authors, year of publication, study design, study population, definitions and reported rate of recurrent/persisting IgAV (only for cohort studies) were extracted and collected on a spreadsheet by one reviewer (JM) to identify the most commonly used definitions. Due to time constraints on this work, quality appraisal of individual studies was not conducted at this stage. However, for its use within the guideline, the quality of included studies will be assessed using the Critical Appraisal Skills Program (CASP) tool specific to the study design (138).

2.2.6. Ethical approval

As per the NHS Health Research Authority (HRA), ethical approval was not required for the current study as it involved anonymous retrospective data collection and review of the existing literature.

2.2.7. Statistical analysis

Clinical and demographical data were compared with the Statistical Package for the Social Science (SPSS) version 27.0 software for Windows (IBM Corp, Armonk, NY, USA). Data were assumed to be non-normally distributed due to the small sample size and the Mann Whitney U test was used for continuous variables. The Pearson's chi-square was applied to categorical variables. A *p*-value of < 0.05 was considered statistically significant.

2.3. Results

2.3.1. Patient selection

Between January 1st, 2015 and December 31st, 2019, a total of 196 children were coded as having a diagnosis of IgAV, of which 29 were coded incorrectly and 14 were considered to have insufficient data. LO and CW identified 16 children who re-presented to our centre outside of the usual HSP nurse-led pathway, of which 13 were further included in the recurring/persisting cohort. The reasons for exclusion and the flow of selecting patients are presented in **Figure 2.1**. Over the 5 years, the recurrent rate was 5.9 % and persisting rate was 2.6 %.

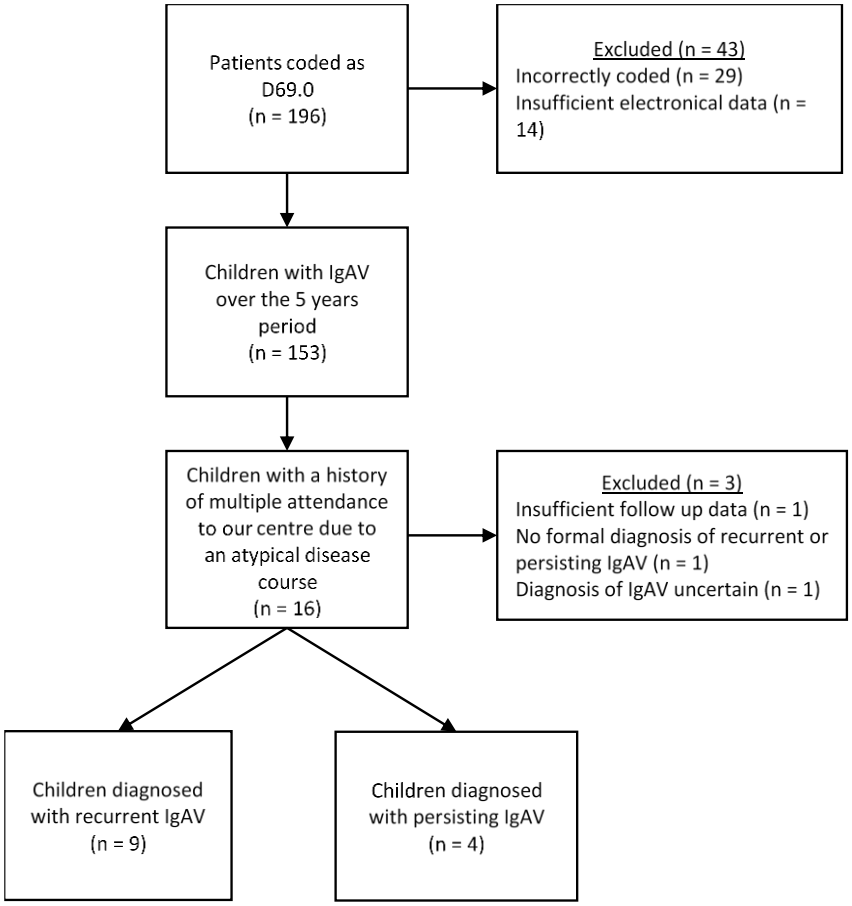


***Figure 2.1**: Flow chart for selection of the cohort.*

2.3.2. Cohort characteristics

The characteristics of the cohort are presented in **Table 2.3**. A total of 4 children were diagnosed as having persisting disease while 9 had recurrent IgAV. The percentage of children that were male was 46% with a median age at presentation of 10.2 years old (range [2.6-15.5]). In comparison, 54% of all the children with IgAV identified from the 5 years cohort (n = 153) were male and the median age was 5.7 years (range [0.6-16.7]). All children initially presented with a rash, which was accompanied by joint involvement in 7 cases and gastrointestinal (GI) involvement in 7 children. The rash was distributed predominantly on the lower limbs in all patients and extended to the upper limbs, trunk and the face in 5, 2 and 1 children respectively. Severe cutaneous involvement, in the form of necrotic and/or ulcerative lesions, was present in two patients, including in the one with a rash extending to the face. None of the patients had renal involvement at presentation. The main reason prompting referral or re-presentation was joint involvement, in the form of arthralgia, arthritis or both, in 9 children. The median time between the first diagnosis of IgAV and a diagnosis of recurrent or persisting IgAV was 18.4 months (range [5.3-150.8]) and this was not significantly different between the groups. A total of seven patients (54%) underwent a skin biopsy to confirm the diagnosis whilst two (15%) had a GI biopsy performed.

	Persisting	Recurrent	Overall	*p*-value
n (%)	4 (31%)	9 (69%)	13 (100%)	-
Male/Female	1/3	5/4	6/7	0.308
Age at diagnosis, years[a]	14.1 [7.9-15.5]	9.1 [2.6-15.1]	10.2 [2.6-15.5]	0.106
Clinical features at first presentation				
Rash[b]	4 (100%)	9 (100%)	13 (100%)	1.00
Distribution				
Lower limbs	4 (100%)	9 (100%)	13 (100%)	1.00
Upper limbs	3 (75%)	2 (22%)	5 (38%)	0.071
Trunk	0 (0%)	2 (22%)	2 (15%)	0.305
Face	0 (0%)	1 (11%)	1 (8%)	0.488
Severe cutaneous involvement	1 (25%)	1 (25%)	2 (15%)	0.522
Gastrointestinal involvement[b]	2 (50%)	5 (56%)	7 (54%)	0.853
Joint involvement[b]	3 (75%)	4 (44%)	7 (54%)	0.308
Primary reason(s) for referral / re-presentation				
Rash[b]	3 (75%)	1 (11%)	4 (31%)	**0.021**
Gastrointestinal involvement[b]	1 (25%)	3 (33%)	4 (31%)	0.764
Joint involvement[b]	3 (75%)	6 (67%)	9 (69%)	0.764
Renal involvement[b]	3 (75%)	1 (11%)	4 (31%)	**0.021**
Persisting proteinuria/haematuria without renal involvement[b,c]	0 (0%)	3 (33%)	3 (23%)	0.188
Symptoms attributable to IgAV at any point during follow up				
Rash[b]	4 (100%)	9 (100%)	13 (100%)	1.00
Gastrointestinal involvement[b]	2 (50%)	4 (44%)	6 (67%)	0.853
Joint involvement[b]	4 (100%)	8 (89%)	12 (92%)	0.488
Renal involvement[b]	3 (75%)	1 (11%)	4 (31%)	**0.021**
Persisting proteinuria/haematuria without renal involvement[b,c]	0 (0%)	4 (44%)	4 (31%)	0.109
Time in months between first diagnosis and diagnosis of recurrent/persisting IgAV[a]	21.0 [5.3-29.4]	13.6 [6.9-150.8]	18.4 [5.3-150.8]	0.940

Table 2.3*: Clinical characteristics of children diagnosed with recurrent or persisting IgAV. [a]n (%); [b]median [range]; [c]renal involvement was defined as a urinary albumin to creatinine ratio > 30 mg/mmol. Significant p-value are highlighted in bold text. Due to rounding, percentages may not add up to 100.*

2.3.3. Potential triggers

One patient had a history of recurrent tonsillitis, one reported having recurrent mouth ulcers and another one had extensive caries at re-presentation. Exercise was reported to trigger flares in three patients, stress in one and cold weather in another patient. Upper respiratory tract infections (URTIs) were found to precede the onset of relapses in four patients. During follow up, the ESR of 8 patients was elevated outside of their age-specific normal range, one had slightly low complement C3 (1.08 g/L) and another one had low C4 titres (0.10 g/L). All of the other patients had normal complement titres, although there was a tendency towards low C4 levels (mean 0.24 g/L; SD ± 0.07). The serum IgA levels were increased above reference range in five children (38%), whilst four (31%) were found to be ANA positive. None of the children in this cohort were ANCA-positive.

2.3.4. Treatment received

The different treatments received by the patients in this cohort is presented in **Table 2.4**. Ten out of the 13 children received analgesia due to IgAV: paracetamol in all cases, followed by non-steroidal anti-inflammatory drugs -NSAIDs- (i.e., ibuprofen and diclofenac) in 4 cases. Five children were treated with opioids to manage IgAV-related pain. All children with persisting disease received corticosteroids (CS), either oral or intravenous (IV), whilst only a third of the recurrent group did. Similarly, all of the children in the persisting group received disease modifying anti-rheumatic drugs (DMARDs). The DMARDs used in this cohort included mycophenolate mofetil (MMF), azathioprine, hydroxychloroquine, dapsone and infliximab. Infliximab and intravenous immunoglobulins (IVIG) were used in a case with severe colitis with abdominal pain, rectal bleeding and vomiting. Two patients with recurrent purpura who received DMARDs (hydroxychloroquine n = 1, dapsone n = 1) did not receive corticosteroids beforehand. One patient in the persisting group underwent a tonsillectomy but this failed to prevent further relapses. The time from referral to initiation of the DMARDs was significantly lower in patients with persisting disease (median 4.3 months; range [1.2-6.1]) compared to the recurrent group, which was more than 2 years post-referral (median 24.3 months; range [13.9-41.4]; p=0.029).

Treatment	Persisting n = 4	Recurrent n = 9	Overall n = 13	*p*-value
Analgesia[a]	4 (100%)	6 (67%)	10 (77%)	0.109
Paracetamol[a]	4	6	10	
NSAIDs[a]	1	3	4	
[b]	Ibuprofen (1)	Ibuprofen (3)	Ibuprofen (4)	
		Diclofenac (1)	Diclofenac (1)	
Opioids[a]	1	4	5	
[b]		Codeine (1)	Codeine (1)	
		Dihydrocodeine (1)	Dihydrocodeine (1)	
		Morphine - oral (2)	Morphine – oral (2)	
	Morphine – IV (1)		Morphine – IV (1)	
Corticosteroids[a]	4 (100%)	3 (33%)	7 (54%)	**0.026**
Oral [b]	Prednisolone - oral (4)	Prednisolone - oral (3)	Prednisolone – oral (7)	
IV [b]	IV pulsed methylprednisolone (1)	IV pulsed methylprednisolone (1)	IV pulsed methylprednisolone (2)	
DMARDs[a]	4 (100%)	4 (44%)	8 (62%)	0.057
MMF	3	-	3	
Azathioprine	-	2	3	
Hydroxychloroquine	-	2	2	
Dapsone	-	1	1	
Infliximab	1	-	1	

Others				
Management of GI involvement / gastro-protection due to CS use [a,b]	1 (25%) Omeprazole (1)	4 (44%) Omeprazole (4) Ondansetron (1) Buscopan (1) Dicycloverine (1) Mebeverine (1)	5 (38%) Omeprazole (5) Ondansetron (1) Buscopan (1) Dicycloverine (1) Mebeverine (1)	0.506
Management of renal involvement [a,b]	2 (50%) Lisinopril (2)	1 (11%) Lisinopril (1)	3 (23%) Lisinopril (3)	0.125
Time in months from first presentation to DMARDs initiation[c]	14.8 [1.8-24.5]	39.0 [23.5-95.4]	24.1 [1.8-95.4]	0.057
Time in months from referral to DMARDs initiation[c]	4.3 [1.2-6.1]	24.3 [13.9-41.4]	10.0 [1.2-41.4]	**0.029**

Table 2.4: *Medications received by children diagnosed with recurrent or persisting IgAV. [a]n (%); [b]drug name (n); [c]median [range]. Significant p-value are highlighted in bold text. NSAIDs: non-steroidal anti-inflammatory drugs. IV: intravenous. DMARDs: disease modifying anti-rheumatic drugs. MMF: mycophenolate mofetil; GI: gastrointestinal; CS: corticosteroids. Due to rounding, percentages may not add up to 100.*

2.3.5. Comparison between patients with and without renal involvement

Patients were then grouped according to the presence of renal involvement, to compare the treatment regimen between the subgroups (**Table 2.5**). Nine patients only suffered from extra-renal manifestations whereas 4 had IgAVN, all of which were biopsy-proven (ISKDC IIIa n = 1; IIIb n = 1; IV n = 1). Of note, one patient evolved from IgAV to IgA nephropathy, they are still included in the cohort and they were scored using the MEST-C score (M-1; E-1; S-0; T-0; C-0). All patients with renal involvement were treated with corticosteroids compared to a third of the patients without. MMF was used in three of the patients with IgAVN, with infliximab in one case, due to gastrointestinal involvement rather than the nephritis. Hydroxychloroquine, azathioprine and dapsone were used to treat the recurring or persisting extra-renal manifestations of IgAV.

	Renal involvement (n = 4)	**No renal involvement (n = 9)**	***p*-value**
Diagnosis			
Recurrent IgAV[a]	1 (25%)	8 (89%)	**0.021**
Biopsy-proven IgAVN[a,1]	4 (100%)	0 (0%)	-
Corticosteroids	4 (100%)	3 (33%)	**0.026**
DMARDs - total[a]	3 (75%)	5 (56%)	0.506
MMF[a]	3 (75%)	0 (0%)	-
Infliximab[a]	1 (25%)	0 (0%)	-
Hydroxychloroquine[a]	0 (0%)	2 (22%)	-
Azathioprine[a]	0 (0%)	3 (33%)	-
Dapsone[a]	0 (0%)	1 (11%)	-
Time in months between first presentation and DMARDs initiation[b]	8.1 [1.8-24.5]	35.3 [21.6-95.4]	0.143
Time in months between referral and DMARDs initiation[b]	2.9 [1.2-6.1]	16.6 [5.6-41.4]	0.071

***Table 2.5**: Comparison of treatment regimen between patients with and without nephritis. [a]n (%) ; [b]median [range]; Renal involvement was defined as a urinary albumin to creatinine ratio > 30 mg/mmol (79). Significant p-value are highlighted in bold text. IgAVN: IgA vasculitis nephritis. DMARDs: disease modifying anti-rheumatic drug; MMF: mycophenolate mofetil. Due to rounding, percentages may not add up to 100.*

Although it was not statistically significant in this small subgroup, there was a trend towards children with IgAVN being treated more promptly with DMARDs than children without renal involvement (p=0.071).

2.3.6. Follow up, disease burden and outcomes

In terms of the disease burden, 5 out of the 13 children were referred to psychology services due to ongoing disease-associated psychological burden. School attendance and feeling self-conscious of the rash and/or frustrated by the refractory symptoms were reported in 6 and 7 children respectively. In addition, more than half of the cohort were admitted at least once and 10 re-presented to the A&E department due to IgAV. Finally, only three children were discharged as of November 21st, 2021, 2 were in remission but remained under follow up and 8 were still followed up due to ongoing disease (**Table 2.6**).

	Persisting (n = 4)	**Recurrent (n = 9)**	**Overall**
Disease burden			
Referred to psychology services due to recurrent/persisting IgAV[a]	2 (50%)	3 (33%)	5 (38%)
School attendance affected[a]	3 (75%)	3 (33%)	6 (46%)
Self-conscious of the rash and/or frustrated by the disease[a]	3 (75%)	4 (44%)	7 (54%)
Follow up			
Months follow up[b]	42.6 [35.0-71.7]	63.8 [14.3-165.7]	57.7 [14.3-165.7]
Admitted at least once due to recurrent/persisting IgAV[a]	3 (75%)	5 (56%)	8 (62%)
Presented to AE at least once due to recurrent/persisting IgAV[a]	3 (75%)	7 (78%)	10 (77%)
Outcome			
Discharged[a]	1 (25%)	2 (22%)	3 (23%)
Still under follow up - remission[a]	1 (25%)	1 (11%)	2 (15%)
Still under follow-up – ongoing disease[a]	2 (50%)	6 (66%)	8 (62%)

***Table 2.6**: Disease burden and outcomes of the cohort. an (%) ; bmedian [range].*

2.3.7. Systematic literature review

The search was conducted on April 25th, 2022 and yielded 1224 results. After duplicates removal (n=458), 766 records were eligible for screening. Of these, the full text of 128 records was assessed for eligibility and 39 studies were included. A further 11 records were identified through references screening of which one paper was eligible, bringing the total to 40 studies included in this literature review. The detailed process of selection is presented in **Figure 2.2** (PRISMA flowchart). Briefly, this systematic review included a total of 13,086 children (7,111 males and 5,975 females) aged 0.2 to 17 years old. Of the studies included, 5 were prospective cohort studies (minimum follow-

up range [0.5-4] years), 33 were retrospective studies, one was cross-sectional and one was a case series (n=8). Four studies were multicentre national studies, one used a nationwide database whereas the remaining studies were all single-centre.

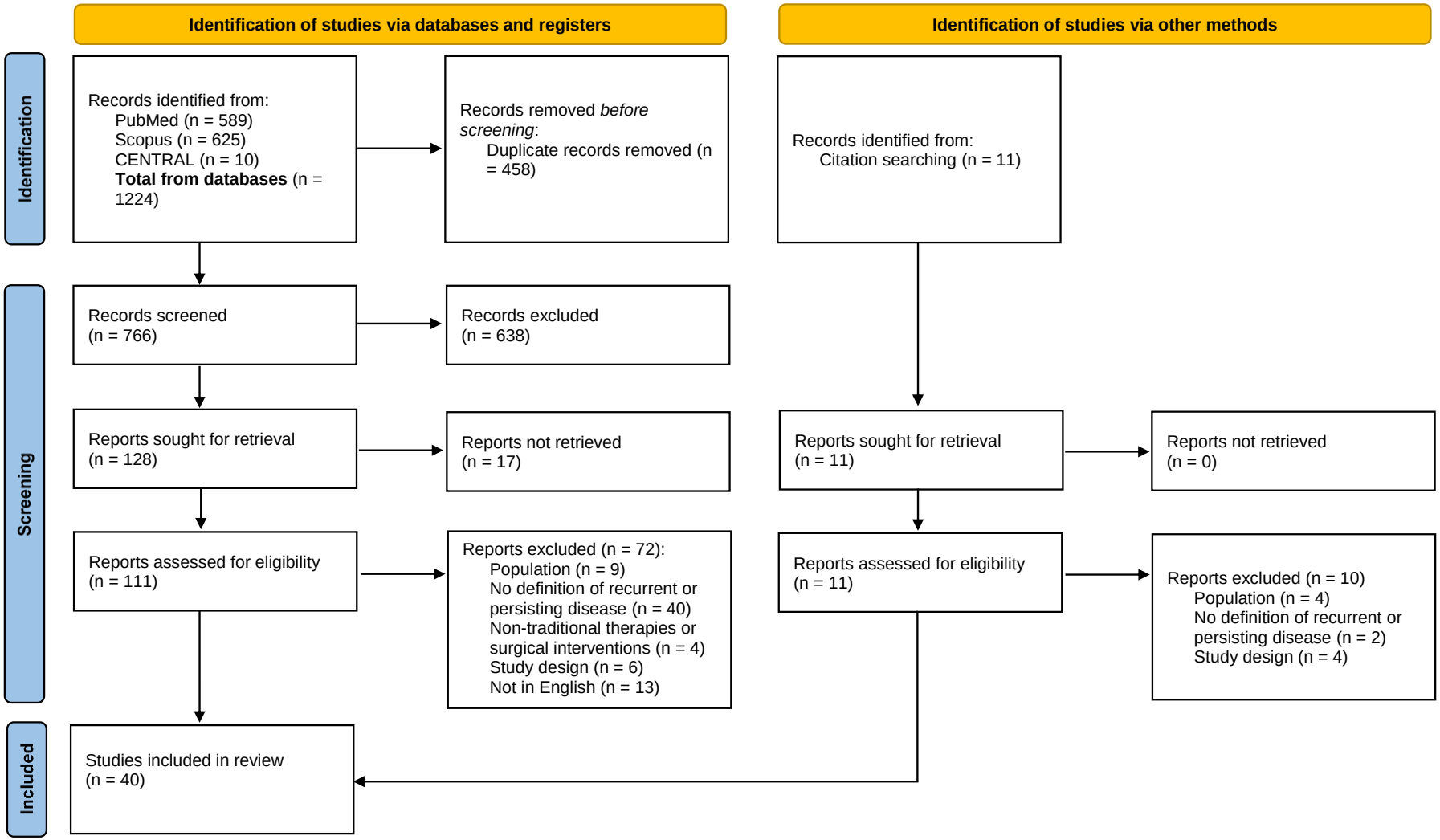


***Figure 2.2**: Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram presenting the process of screening and selection, from Page et al (134).*

2.3.7.1. *Recurrent disease*

A total of 34 papers provided a definition for recurrent IgAV in children, the full definitions can be found in **Appendix 3**. Most studies considered that patients with a prior diagnosis of IgAV needed to be asymptomatic for a specific period of time and that the resolution of all symptoms from the initial episode was required prior to the new presentation to be considered a recurrence or a relapse. The reappearance of the purpuric rash was a mandatory criterion in one study (90) and another mentioned obtaining a "second diagnosis of HSP" (14), thus implying the mandatory rash alongside any other characteristic signs in line with the EULAR/PRINTO/PReS criteria (79). The remaining definitions were less specific and described recurrence/relapses as the reappearance of the characteristic cutaneous lesions or other systemic manifestations characteristic of IgAV. Discontinuation of all medications for at least 3 months, 1 month and 2 weeks was required in three studies (12, 139, 140) in addition to the disappearance of symptoms. Teng and colleagues defined a relapse as the recurrence of clinical signs/symptoms requiring resumption of immunotherapy or an increased dose (141) whereas Farisogullari *et al.* considered that the resumption of corticosteroids or other immunosuppressive therapies, or a significant increase in glucocorticoid dose (by at least 50%) was necessary to qualify as a relapse (142). The reappearance of symptoms following remission was mentioned in four studies, the definitions used for remission will be discussed in **2.3.7.4**.

Out of the 34 studies, the majority (n=19) considered that a 4 weeks symptom-free period was required between episodes, whereas 6, 6, and 1 studies used 2 weeks, 3-months, and 6-months respectively. Two studies did not specify any asymptomatic period. The mean recurrence rate across all studies for which it was reported (n=28) was 18.1% ± 14.1 (range [4.0-66.0]). The mean recurrence rate did not statistically significantly differ according to the symptom-free interval between two presentations used (p=0.533), although greater variability was observed in papers using 2 weeks as a definition (SD 22.8%) (**Table 2.7**).

Asymptomatic interval	**2 weeks**	**4 weeks**	**3 months**
Number of papers, n	6	17	5
Children, n	5128	3490	1930
Mean recurrence rate ± SD	25.4 ± 22.8	17.2 ± 11.5	12.2 ± 5.1

***Table 2.7**: Mean recurrence rates reported by studies depending on the asymptomatic interval used. Data are presented as mean ± standard deviation (SD).*

2.3.7.2. *Persisting disease*

Persisting or refractory IgAV was defined by 5 studies, the definitions are presented in **Table 2.8**. Contrary to the recurrent definitions, the definitions of persisting disease were heterogenous. Symptoms characteristic of IgAV had to persist for more than 6 months after the onset of the initial

episode in one study (12) and 12 months in another (96). The persistence of the rash for at least 3 months was a criterion in one study (143). The lack of response to treatment was a criterion in two studies (109, 142) whereas failure to achieve complete remission was necessary in another (12).

Author. *Year*	**Definition used**	**Time criterion**
Alfredo, C. S. *et al*. *2007 (96)*	Cases were defined as chronic when cutaneous, abdominal and renal manifestations persisted for a period of 12 months or more.	12 months
Crayne, C. B. *et al*. *2018 (109)*	Refractory HSP was defined as recurrence of symptoms following taper of CS. Refractory HSP was defined as CS dependent and/or DMARD refractory disease.	NA
Farisogullari, B. *et al*. 2*022 (142)*	Refractory: lack of response to standard dose corticosteroids alone or in combination with other immunosuppressive therapies	NA
Liao, C. H. M. *et al*. *2020 (12)*	Refractory disease was defined as not achieving complete remission 6 months after disease onset.	6 months
Schinzel V. *et al*. *2019 (143)*	Chronicity was defined as the persistence of cutaneous alterations for at least three months.	3 months

Table 2.8*: Definitions of persisting IgAV in children from the systematic literature review. HSP: Henoch Schönlein purpura; CS: corticosteroids; DMARD: disease modifying anti-rheumatic drug.*

2.3.7.3. *Recurrent/persisting purpura*

Some papers defined recurrent purpura or persisting purpura instead of recurrent/persisting IgAV, hence these definitions were recorded separately. One paper reported recurrent purpura as new cutaneous lesions after total recovery (144). All papers defining persistent purpura agreed in the definitions they used, which was a purpuric rash typical of IgAV lasting for over 4 weeks (**Table 2.9**).

2.3.7.4. *Remission*

Although remission was not included in the search, the definitions of remission were recorded as a secondary outcome. Three studies provided definitions for remission in paediatric IgAV. The authors of the case series that included 8 patients with chronic-steroid dependent IgAV treated with rituximab considered that no active rash, arthritis, nephritis (haematuria and proteinuria), or gastrointestinal distress following treatment with rituximab was required (109). Liao *et al.* defined remission as the resolution of skin purpura, arthralgia/arthritis, and abdominal pain, combined with normal renal function and absence of proteinuria and haematuria, as well as discontinuation of all medication (12). Finally, Farisogullari and colleagues defined remission as a Birmingham Vasculitis Activity Score (BVAS) of 0 (142), although the same definition was applied to both adults and children.

Author. *Year*	**Definition used**	**Time criterion**
Buscatti, I. M. *et al*. *2018 (144)*	Persistent purpura/petechiae, as skin lesions persisting for ≥ 1 month.	4 weeks
De Almeida, J. L. J. *et al*. *2007 (145)*	Persistent palpable purpura lasting for more than 1 month after disease onset.	4 weeks
Dundar, H. A. et *al*. *2020 (99)*	Persistent purpura was defined as skin involvement persisting for ≥ 30 days.	4 weeks
Rigante, D. *et al*. *2005 (97)*	Rash persistent over more than 1 month.	4 weeks
Sano, H. *et al*. *2002 (146)*	Persistent purpura was determined as purpura persisting over 1 month.	4 weeks
Sestan, M. *et al*. *2022 (130)*	Persistent purpura was considered to be purpura persisting for ≥1 month.	4 weeks
Shin, J. I. *et al*. *2006 (8)*	Recurrent purpura persisting for 1 month or more was classified as persistent purpura.	4 weeks
Zhao, Y. L. *et al*. *2015 (98)*	Persistent purpura was defined recurrent purpura persisting for 1 month or more.	4 weeks

Table 2.9*: Definitions of persisting purpura in children from the systematic literature review.*

2.4. Discussion

The current study described a cohort of children with an atypical course of IgAV. The most common symptom prompting re-presentation was joint involvement and we report rather long times from the first diagnosis of IgAV to a diagnosis of persisting/recurrent disease and initiation of treatment. In addition, a systematic literature review was conducted to identify the most commonly used definitions for recurring and persisting disease, in the absence of any standardised definitions.

2.4.1. Risk factors for an atypical disease course

Persistent purpura is a risk factor for the development of IgAVN (7), although it does not necessarily correlate with poorer outcomes (11). In addition, a recent multicentre study reported that patients with a generalised rash, necrotic or ulcerative skin lesions, or a rash extending to the upper limbs had higher rates of relapse compared to children with a purpura confined to the lower limbs (130). In the current series, all patients exhibited a rash with lower limb predominance at presentation and 5 (38%) also demonstrated an upper limb distribution. Of these, the rash extended to the trunk in one patient and the trunk and the face in another, which was associated with severe skin lesions. If a more extensive or severe rash may predispose to a more complex disease course, data from much larger cohorts is needed to confirm any association.

Large cohort studies have investigated the potential risk factors associated with relapsing IgAV, yet results are inconsistent across studies. Increasing age at onset (usually over 8 years old) was identified as a risk factor by several studies (8, 9, 12, 92) whilst others did not find this association (12, 14, 67, 93, 94, 97). In one study, the optimal threshold to predict refractory IgAV was age ≥ 7.2 years old at presentation (sensitivity 69.7%, specificity 70.1%, area under the curve -AUC- 0.73) (12). In the current series, median age was 10.2 years old (range [2.6-15.5]) whilst the median age of the 5-year cohort of 153 children with IgAV was 5.7 years (range [0.6-16.7]). This supports that older age at presentation may be associated with an atypical disease course, although a young age at presentation is not protective as the youngest patient in our cohort presented aged 2.6 years old. CS treatment was also reported as a risk factor for recurrent IgAV (12, 14, 93, 94). Since a more severe form at presentation would require CS therapy, it may represent a possible confounder for CS, as suggested by Jauhola and colleagues, who did not find any association between corticosteroid therapy and HSP recurrences (9).

Regarding laboratory markers, Trapani and colleagues found that higher ESR values were correlated with relapses (93) but other studies did not find any correlation between recurrence and laboratory markers levels (92, 94, 139). The elevated ESR in 8 of our patients is not unexpected because of the long-standing inflammation and has been reported in children with IgAV before (147).

Likewise, increased IgA levels in 5 of our patients was not unexpected (37). White blood cell levels, haemoglobin levels and ANA positivity were significantly different between patients with and without recurrences in one study but they could not predict the outcome by multivariate analysis (94). In our cohort, four patients (31%) were ANA-positive however the larger cohort did not routinely have ANA measured and up to 15% of healthy children have a positive ANA test in previous studies (148, 149), although Calvo Rio and colleagues did report a significantly increased incidence of ANA-positivity in a large cohort of children with relapsing IgAV compared to children without relapses (27.3% vs 8.3%) (94). C3 and C4 serum levels are not recognised clinically useful markers of IgAV disease activity. Our findings of only one patient with low C3 and another with low C4 does not support their use as a marker for recurring/persisting disease, although C4 levels tended to be on the lower side of the age-specific range for this group.

Severe bowel angina (8), arthritis (92, 94) GI involvement (94, 95) and involvement of the skin, joints, abdomen and kidneys altogether at first presentation (139) were also reported to be risk factors by individual studies for a complicated disease course. Chronic tonsillitis and other ENT-related problems may be associated with recurrent purpura (35), although only three children from our cohort were reported to suffer from such problems. It is interesting that exercise seemed to induce flares in three of the patients in our series, as limitation of strenuous exercise helped to reduce the recurrence rate in children with IgAV, in one recent study (150). Finally, obesity was strongly associated with persistent purpura lasting for more than 4 weeks in one study with an odd-ratio of 3.2 and it was suggested that increased adipose tissue may contribute to disease severity (99). This may warrant exploration in further studies, as we did not collect the body-mass index of our cohort.

2.4.2. Treatment

Despite being self-limiting and only requiring supportive treatment in the majority of patients, IgAV can be severe and opioid analgesia may be required (151), which we demonstrated in 5 out of 13 patients. In our cohort, the treatment of the nephritis patients was in line with the recently published SHARE initiative guideline (Single Hub and Access point for paediatric Rheumatology in Europe) (78), which recommended oral prednisolone first line followed if necessary by IV pulsed methylprednisolone and MMF (78). However, there is no standardised treatment algorithm for children presenting with recalcitrant extra-renal manifestations and a striking lack of high-quality evidence on how best to treat these patients.

In addition to their use in nephritis, the SHARE guideline recommends using CS in severe abdominal pain and rectal bleeding, as it may reduce the intensity and duration of the episode, although the consensus panel noted the lack of robust data to support this recommendation (78). No

other recommendations were issued regarding the use of corticosteroids in refractory skin, joint or abdominal involvement, but it will usually be the first-line treatment prescribed by clinicians for induction of remission (151). Patients with severe symptoms may benefit from early CS treatment, as prednisone was shown to reduce extrarenal symptoms and improve clinical outcomes in these cases (9, 152, 153). Severe skin involvement, with bullous or necrotic lesions, has been successfully treated with glucocorticoids in case reports (113, 114, 154), but the efficacy of this treatment for mild purpura recurrence or persistence remains unknown. In our cohort, all of the children with persisting IgAV and a third of the recurrent group received CS, with oral prednisolone used first-line which was followed if necessary by IV pulsed methylprednisolone. In two patients, DMARDs were used without a preceding course of CS. However, repeated or long-term use of CS should be minimised due to the high frequency of adverse events (155) and other immunosuppressive agents need to be considered.

Azathioprine, a purine synthesis inhibitor (156), was used in two patients with extra-renal manifestations, and this was supported by a case series reporting 6 children with steroid-resistant extra-renal IgAV successfully treated by azathioprine (111). For the treatment of chronic or recurrent cutaneous manifestations of small vessel vasculitides, colchicine and dapsone are usually recommended first followed by MMF and azathioprine second line, but this is not specific to IgAV (157, 158). Dapsone, an anti-leprosy drug, was used in two of the patients with recurrent disease and a systematic review concluded it may be an effective treatment for recalcitrant IgAV but this was mainly based on data from case reports (159). A clinical trial is currently underway to investigate the efficacy of dapsone, colchicine and azathioprine in skin-limited vasculitis, including adult patients with IgAV (160) (ClinicalTrials.gov; NCT02939573). Hydroxychloroquine, an antimalarial drug, is already used for systemic autoimmune rheumatic diseases and is recommended for cutaneous small vessel vasculitis not responding to conventional treatment (158). To treat IgAV, it is sometimes used in clinical practice and 2 patients from our series were prescribed it, although there is no evidence of its efficacy (161, 162). Both dapsone and hydroxychloroquine are safe drugs, available in tablet formulation, inexpensive compared to other immunosuppressive agents and still routinely used despite the lack of evidence supporting their efficacy in IgAV. Finally, one patient received infliximab and IVIG to treat severe colitis secondary to vasculitis. Interestingly, the anti-TNF monoclonal antibody infliximab is also thought to possibly trigger IgAV (38, 44) and we were unable to find any other case in the literature where infliximab was used. IVIG are very rarely used, but the few case reports in the literature did report successful treatment of severe gastro-intestinal involvement (163-166). Although they were not used in our cohort, methotrexate and rituximab may also play a role in treating refractory paediatric IgAV (108, 167, 168). Finally, tonsillectomy was successful at treating severe proteinuric IgAVN in small series of paediatric patients (169-172) and it was suggested that it may

alleviate extra-renal symptoms in children with a prolonged disease and concomitant tonsillitis or infectious ENT foci (35, 169). Nonetheless, these findings were based on very small uncontrolled cohorts, and a large multicentre European study including 1,147 adult patients with IgA nephropathy found no benefit of tonsillectomy in improving the renal outcomes (173). One patient from our series underwent a tonsillectomy, for recurrent tonsillitis, which failed to prevent further IgAV-related disease activity. Due to its invasive and aggressive nature and despite the lack of robust evidence, this procedure may still be considered in a small select group of patients with coexisting IgAV and underlying chronic ENT infections (19).

As demonstrated above, the rather small body of evidence regarding treatment of these cases relies mainly on case reports or small case series. There is an urgent need for standardisation of care in order to provide foundation for randomised clinical trials.

2.4.3. Standardising care in childhood IgAV

One of the many challenges in IgAV lies in the absence of a validated scoring tool to assess disease activity and severity. The Paediatric Vasculitis Activity Score (PVAS) is considered to be the gold standard to measure disease activity in paediatric systemic vasculitides, however it has not been validated for use in IgAV (124). The adult version of this score, the Birmingham Vasculitis Activity Score (BVAS), has been successfully evaluated in a preliminary study in a large cohort of children with systemic vasculitis, including 669 (84%) with IgAV (174) and has been used to define remission in children with IgAV (142). Having an efficient and valid scoring tool is essential to be able to set core outcomes to assess disease activity and response to therapy. As an example, clinical trials have been relying on the BVAS score in order to define relapsing or persisting disease in adult patients with ANCA-associated vasculitis (AAV) (175-179), as advised by the 2006 EULAR recommendations for conducting clinical trials in systemic vasculitis (180).

According to PVAS, persistent symptoms are items that have been present for more than 4 weeks but less than 3 months (124). The PVAS itself does not provide any definition for relapse but its use is well established to define remission and hence relapsing / persisting disease in childhood vasculitis (181-184). With neither the PVAS and BVAS being validated to use in children with IgAV, a previous student (CW) worked on the preliminary validation of a new score, the IgA-VAS, to monitor disease activity children with IgAV. The IgA-VAS performed well, although further optimisation and refinements were needed alongside prospective validation (131). Validation of this score in paediatric IgAV may allow for the identification of a threshold which could contribute to the definitions of recurring and persisting disease, in a similar way to AAV.

2.4.4. Proposed definitions

There is no universally agreed definition for recurrent or persisting IgAV in children and discrepancies in the definitions used in the literature, as demonstrated by the systematic literature search conducted within this chapter. The majority of reports considered a recurrence or a relapse of IgAV when an asymptomatic patient presented characteristic IgAV symptoms at least 4 weeks after being symptom-free from the initial episode. It has been widely demonstrated that relapses most often occur within 6 months of first presentation (9, 14, 93-95), however it is unclear what the cut-off should be to distinguish manifestations from the initial episode to new manifestations suggestive of disease recurrence. The reported recurrence rate greatly varies in the literature and was reported to range from 2.6% to 66% (9, 12, 14, 90-93) and it was suggested this may be due the differences in the definitions used allowing recurrence rates to be overestimated (14). We did not find any significant association between the asymptomatic interval and the rate of recurrence. Given that the majority of authors chose 4 weeks as a cut-off and large epidemiologic studies have demonstrated that relapses often occur 1 month after the first episode (92, 94, 185), it seems reasonable to suggest that 4 weeks after the resolution of symptoms is appropriate for consideration in the definition. Only two reports required the mandatory criterion of the purpuric rash alongside any other symptoms to qualify for a recurrence, however, it can be assumed that the "classical signs and symptoms of IgAV" mentioned by authors refer to the widely accepted EULAR/PRINTO/PReS criteria (79) and therefore, this diagnostic criteria should be applied to diagnose any relapse of the disease. This is also supported by the current case series where all children being diagnosed with recurrent or persisting IgAV re-presented with the classical IgAV rash alongside any other symptoms aforementioned. Hence, based on the literature, we propose to define a relapse of IgAV as a new onset of typical purpura alongside any other characteristic signs of IgAV (i.e., abdominal pain, joint involvement, renal involvement, in line with the EULAR/PRINTO/PReS criteria (79)) at a time 4 weeks after the complete resolution of previous symptoms.

Very few reports defined persisting disease. However, several studies defined persistent purpura as a persisting rash lasting for over 4 weeks. In keeping with the definition of recurrent IgAV proposed above, the points previously discussed and the PVAS definition of persisting symptoms (124), we propose persisting IgAV to be defined as a typical purpura alongside any other characteristic signs of IgAV (as per EULAR/PRINTO/PReS (79)) lasting over 4 weeks.

These proposed definitions will be discussed in the aforementioned UK IgAV guideline development group to gather expert consensus.

2.4.5. Areas of unmet needs

The psychological burden of a prolonged course of IgAV is unrecognised. The fact that half of the patients in the present series were referred to psychology services and that more than a third reported feeling frustrated by the disease course is important to consider. In addition, these numbers are very likely to be underestimated as it was retrospective data collection. Adolescence is a critical period for psychosocial development and health complications, especially skin conditions, may be challenging at this time when self-image and self-confidence are perceived to be important (186). Children with a prolonged IgAV course may not be able to participate in physical education or other extra-curricular activities, therefore, a feeling of exclusion may arise. In addition, IgAV is perceived as self-limiting, as the majority of patients follow this disease course, adding frustrations and potential delays in specialist care. The care of these complex patients is also disseminated between paediatric nephrologists, rheumatologists, dermatologists and gastroenterologists (129). Finally, the rather long time to obtain a diagnosis, initiate treatment or the need for further invasive tests (i.e., skin biopsy) reported in the current study further reinforce the need for more efficient diagnostic and monitoring tools alongside improved communication between specialists. All of these factors may be distressing and frustrating for patients and their families, as reflected in this series and in a recent study that found that parents' resilience (i.e., the ability to cope with stress and functioning well) was negatively correlated to the frequency of recurrences of their child's IgAVN (187). This warrants further research and emphasises the importance of a holistic approach: atypical cases may benefit from early psychological input in order to identify the needs of both patients and their families to provide support at an early stage.

2.4.6. Limitations

This study has several limitations. First, there are obvious limitations associated with the retrospective data collection. Data were collected by only one author (JM) and about half of the cohort did not initially present to Alder Hey but to their local district hospital/GP. This meant data were gathered from the referral letters so some data were not retrievable, especially the full history and investigations performed at first presentation. In addition, assumptions were made for the rash distribution at presentation or the analgesia received. Another limitation are the definitions used which were relatively stringent and patients who represented to A&E with probable relapses but without a formal diagnosis by a paediatric rheumatologist or nephrologist were not captured. This may explain why we report such a low recurrence rate compared to what is usually reported in the literature. We were unable to quantify the exact number of recurrences or any symptom-free intervals from the medical records due to patients presenting to their GP, local district hospital or managing the relapses at home. This study could have benefited from a disease-control group including patients

with an uncomplicated IgAV course, in order to identify any risk factors associated with atypical disease, hence it is difficult to assess whether children from our series have significantly different findings when compared to those with a typical disease course. Finally, risk of bias of the studies included in the systematic literature review was not assessed due to time constraint on this work.

2.5. Conclusions

There is no universally agreed definition of recurring/persisting IgAV in children and this may contribute to a delay from first presentation to diagnosis and/or treatment, as suggested in this case series. The lack of standardisation most certainly hinders the feasibility of clinical trials, which are urgently needed to provide robust evidence on how best to manage these cases. We propose to define recurrent IgAV as 'a new onset of typical purpura alongside any other characteristic signs of IgAV (as per EULAR/PRINTO/PReS criteria) at a time 4 weeks after the complete resolution of previous symptoms' and persisting IgAV as 'a typical purpura alongside any other characteristic signs of IgAV (as per EULAR/PRINTO/PReS criteria) lasting over 4 weeks', although expert consensus is warranted to confirm these. Further research is needed to study this subgroup of children with an atypical disease course, as evidence is greatly lacking.

3. Urinary IgA and C5a and renal involvement in children with IgA vasculitis

3.1. Introduction

Renal involvement accounts for the majority of the long-term mortality and morbidity in children with IgAV, hence the need for all children to undergo a 6 month follow-up period of renal monitoring to identify any evolving nephritis (19). Currently used clinical tools, the gold-standard renal biopsy, which remain invasive, and proteinuria have limitations (126). Hence, there is a need for improved non-invasive biomarkers offering real-time disease activity evaluation. The first phenomenon thought to lead to IgAVN is the mesangial deposition of galactose-deficient IgA1-containing immune complexes in the glomerulus (44). However, the pathophysiology of IgAVN and why some children develop significant renal involvement remains unknown. As a result, a special interest is given to this immunoglobulin, and determining if it can be detected in the urine, a non-invasive biofluid, and how it correlates to clinical characteristics could lead to further studies to assess its prognostic value and to characterise any relationship between IgA glycan-specificity and the renal phenotype.

In addition, there is growing evidence supporting the role of the complement system in IgA-mediated renal diseases (44, 50) as mentioned in introduction section **1.1.3.5**, with several selective complement inhibitors drugs currently under evaluation for the histologically similar disease, IgA nephropathy (188). However, limited data is available regarding complement activation in IgAVN apart from histological evidence of complement component deposits in the glomerulus (55, 56, 189).

There is a need for improved understanding of the pathophysiology of IgAV and the biological pathways leading to nephritis, which remain largely unknown.

3.1.1. Aims

The primary aim of the research described in this chapter was to assess whether urinary IgA and C5a could be detected in children with IgAV and to identify any relationship with nephritis. The secondary aim was to compare the serum IgA concentrations between the groups and assess any correlation to the urinary IgA levels.

3.2. Materials and methods

3.2.1. Patient selection and definitions

Children were recruited as part of the IgA Vasculitis study. Children of any sex aged < 18 years old at first presentation with a diagnosis of IgAV according to the EULAR/PRINTO/PReS 2008 Ankara-

endorsed criteria (79) were eligible to take part. Exclusion criteria were as follows: (i) diagnosis of IgAV uncertain; (ii) other concurrent inflammatory or renal condition; (iii) undergoing dialysis; (iv) no urine sample available in the biobank. This study was cross-sectional so no minimum follow-up was required and a urine sample could be obtained at any point during the follow up.

Patients were grouped according to the presence of renal involvement, into either IgAVN (IgAV nephritis group) or IgAVwoN (IgAV without nephritis group). IgAVN was defined as a urinary albumin to creatinine ratio (UACR) of > 30 mg/mmol at the time of sampling (79). Any renal histology was graded according to the International Study of Kidney Disease in Children (ISKDC) classification for IgAVN (83). Hypertension was defined as a systolic blood pressure above the 95th centile for the child's age, sex and height for < 16 years old or >140 mmHg for children 16 years and older (190).

Healthy controls (HCs) were recruited to provide age and sex-matched urine samples as per the IgA Vasculitis study protocol. They were children (aged < 18 years old) with no relevant past medical history (i.e., no history of autoimmune or renal disease) and not taking any regular medication attending for day-case investigations or surgery.

3.2.2. Data collection

Demographics and clinical data were collected at the time of the sample collection to provide baseline clinical characteristics. This included sex, age, ethnicity, blood pressure, height, serum creatinine (if available), UACR, renal histology report and any medication. A UACR of 0 mg/mmol was assumed for patients with a urine dipstick negative for protein. For one patient for which the MEST-C classification was used on the histological report, the conversion to an equivalent ISKDC grade was performed by consulting a paediatric nephrologist (LO).

3.2.3. Sample processing

Biological samples were processed in accordance with a standard operating procedure. Healthy control urine samples were tested for bacterial contamination using urine dipstick testing and they were discarded if they demonstrated positivity for leukocytes, nitrites, blood or > +1 for protein. Urine and serum samples were centrifuged at 300×g for 10 mins, transferred into a new falcon tube, centrifuged again at 300×g for 10 mins and aliquoted into 1mL (urine) and 200µL (serum) sterile Eppendorf tubes for storage. Samples were stored at -80 °C and were thawed at room temperature on the day of the experiment and vortexed for 10 secs immediately before use. For this experiment, a mix of never-thawed and freeze-thawed urine samples were used for the IgA quantification whereas only samples that had never been thawed were used for the C5a quantification.

3.2.4. Assays

The determination of serum IgA and urinary IgA and C5a concentrations was performed using commercially available enzyme-linked immunosorbent assay (ELISA) kits (Bio-Techne, Abingdon, UK) as per the manufacturer's instructions.

3.2.4.1. *Optimisation of the dilution factor for the total IgA quantification in urine*

First, the IgA ELISA kit was run on 2 samples (IgAV, n=1; HC, n=1) as per **3.2.4.2** to identify the optimal dilution of urine. The manufacturer suggests a 10 to 100-fold dilution with recommendation of 20x dilution. A 10, 20, 50 and 100-fold dilution were used on the urine samples. At the time of the range finding experiment, the clinical data on the cohort were not collected, hence the renal status of the IgAV sample used was unknown. It was later found this sample had a negative urine dipstick at sampling so it was categorised as part of the IgAVwoN group. The best yield was obtained with the 10-fold dilution, suggesting this was the most appropriate dilution factor to use (**Figure 3.1**).

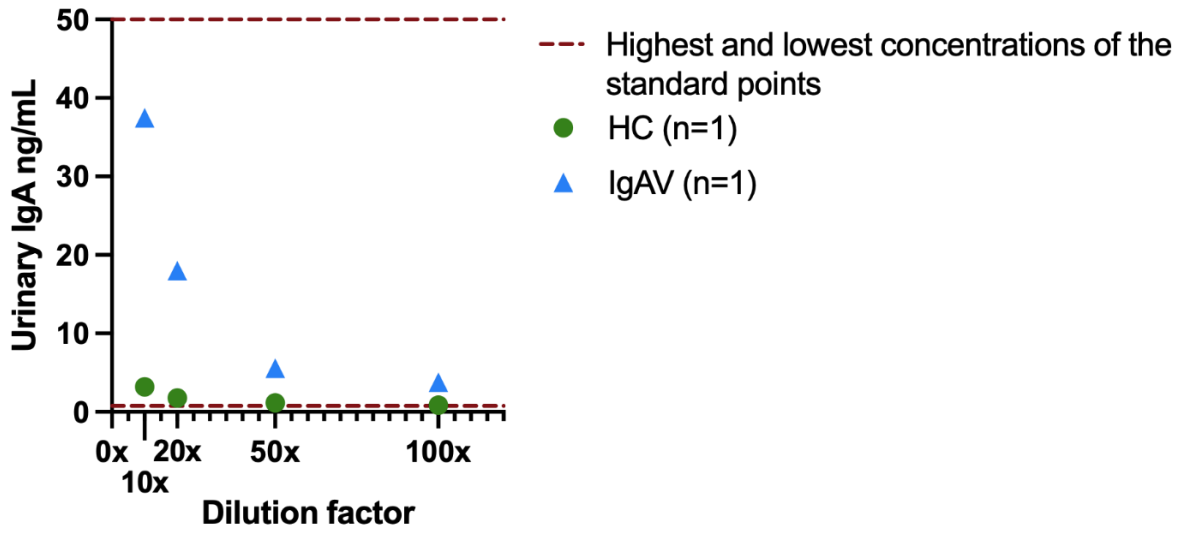


***Figure 3.1**: Range finding experiment results. The red lines represent the highest and lowest concentrations of the standard points used for the manufacturer's recommended standard curve (0.781-50.0 ng/mL). Results are not corrected for urinary creatinine.*

3.2.4.2. *IgA ELISA*

A 50,000-fold dilution was used on the serum samples and a 10- fold dilution on the urine samples, as suggested by the results from the range finding experiment. First, 50µL of samples or standards were loaded in duplicates onto each well and left to incubate for 2 hours at room temperature (RT). The plates were then washed, incubated with 50µL of IgA detection antibody for one hour at RT, washed again and loaded with 50µL of streptavidin-peroxidase (SP) conjugate. After 30 mins of incubation at RT, the plates were loaded with 50µL of chromogen reagent and left to incubate for 6 mins. Following the addition of the stop solution, the plates were read on a microplate reader at 450nm with 570mm wavelength correction on the POLARstar Omega device (BMG LABTECH

GmbH, Ortenberg, Germany). The serum IgA ELISAs were conducted by a previous student (SE) and their supervisor (AJC).

3.2.4.3. *C5a ELISA*

First, the plates were incubated with diluted C5a capture antibody overnight at RT. They were then washed, incubated with 200µL of phosphate buffered saline + 1% bovine serum albumin (PBS + 1% PSA) for one hour at RT, washed again and loaded with 50µL of thawed urine or standards in duplicate. No dilution was performed on the urine samples to perform this ELISA. Following a two-hour incubation period at RT, the plates were washed, coated with 50uL of detection antibody, and incubated for a further two hours. The plates were washed, streptavidin-HRP was added and left to incubate for 20 mins (RT). After one last wash, they were incubated with 3,3',5,5'-tetramethylbenzidine reagents for 20 mins. Following the addition of the stop solution, the plates were read on a microplate reader at 450nm with 570mm wavelength correction on the POLARstar Omega device (BMG LABTECH GmbH, Ortenberg, Germany). The C5a ELISAs were run by two laboratory technicians (SN and RC).

3.2.5. Creatinine quantification

Urinary concentrations of IgA and C5a were corrected for the urinary creatinine concentrations. Automated quantification of urinary creatinine was performed by the Biochemistry Department (Alder Hey Children's NHS Foundation Trust, Liverpool, UK) using a previously described enzymatic creatinine method (191) on the Abbott Architect Ci8200 (Abbott, Illinois, USA).

3.2.6. Ethical approval

All procedures involving human subjects were conducted in accordance with NIHR Good Clinical Practice, HTA Codes of Practice, the Declaration of Helsinki and comparable ethical standards. This study was part of the IgA Vasculitis study which was approved by HRA and Health and Care Research Wales (HCRW) on June 21, 2019 (REC 17/NE/0390, protocol UoL001347, IRAS 236599). Written informed consent was obtained from parents and children prior to any study-related procedure.

3.2.7. Data analysis

The MARS Data Analysis Software version 3.32 for Windows (BMG LABTECH GmbH, Ortenberg, Germany) was used for the data analysis. The difference in optical density (ΔOD) of the 450m and 570nm blank-corrected readings was calculated and a standard curve was generated using the standard concentrations on the x-axis and the corresponding ΔOD on the y-axis. The best fit-line was determined using a four-parameter fit logistic curve-fit (serum IgA, urinary C5a) or linear regression (urinary IgA). The sample concentrations were determined from the standard curve and

results were exported onto Microsoft Excel version 2019 for Windows (Microsoft Corporation, London, UK). The mean value, standard deviation (SD) and coefficient of variation of the duplicates were calculated and corrected for dilution. Values that were below the lowest concentration of the standard points were imputed as (standard lowest concentration)/ √ 2 following previously published convention (192). Data are presented as median [range] unless stated otherwise.

3.2.8. Statistical analysis

The Statistical Package for the Social Science (SPSS) version 27.0 software for Windows (IBM Corp, Armonk, NY, USA) and GraphPad Prism version 8.0 for Windows (GraphPad Software, San Diego, CA, USA) were used for the statistical analysis. The data distribution was assessed using the Shapiro-Wilk test. The student t-test or one-way ANOVA with Tukey's post-hoc test were used for normally distributed data whereas the significance of non-normally distributed data was evaluated with the Mann-Whitney U test or Kruskal-Wallis with Dunn-Bonferroni post-hoc test. The Pearson's chi square test was applied to continuous variables. Linear correlation was assessed with the Pearson's correlation coefficient: a coefficient of < 0.30 was considered negligible, 0.30-0.50 low correlation, 0.50-0.70 moderate correlation, 0.70-0.90 high correlation and 0.90-1.00 very high correlation (193). Receiver operating characteristic (ROC) curves were generated to evaluate the ability of these urinary proteins to discriminate between patients with and without renal involvement. An area under the curve (AUC) of < 0.7 was considered non-discriminant, 0.7-0.8 acceptable, 0.8-0.9 excellent and > 0.9 outstanding (194). A logistic regression model was built to first evaluate the diagnostic values of IgA and C5a together and then with adding any statistically significant demographic/clinical variable into the model; the Hosmer-Lemeshow test was used to assess goodness of fit. A *p*-value of < 0.05 was considered statistically significant.

3.3. Results

3.3.1. Paediatric cohort

A total of 59 children were recruited in this study (IgAVN n=11, IgAVwoN n=36, HCs n=12); their baseline characteristics are presented in **Table 3.1**. The median age of the cohort was 7.1 years old (range [1.8-17.9]) and 66.1% of the cohort were male. Children with IgAVwoN were significantly younger (6.2 years old [1.8-17.2]) than patients in both the IgAVN (11.3 years old [4.4-17.9]; *p*=0.039) and HCs groups (9.6 years old [5.6-14.7]; *p*=0.021). A significantly higher proportion of children within the IgAVN group had hypertension compared to IgAVwoN and there was no statistically significant difference in the serum creatinine levels. Within the IgAVN group, 7 had a renal biopsy demonstrating IgA-positive staining on immunofluorescence (IF). Of these, C3, IgG and IgM positivity were present in six, two and one patient respectively. All had normal renal function, normal complement titres and no

evidence of peripheral oedema. One patient had IgAVN previously demonstrated histologically (focal glomerulonephritis with < 50% crescents – ISKDC grade IIIa) prior to sampling; however, at study baseline, the UACR was less than 30 mg/mmol, hence for the purposes of this data they were categorised as IgAVwoN.

	Overall	IgAVN	IgAVwoN	HCs	*p*-value
n (%)	59 (100)	11 (18.6)	36 (61.0)	12 (20.3)	-
Male[a]	39 (66.1)	6 (54.5)	24 (66.7)	9 (75.0)	0.581
Age, years[b]	7.1 [1.8-17.9]	11.3 [4.4-17.9]$^{+}$	6.2 [1.8-17.2]*	9.6 [5.6-14.7]	**0.004**
Weeks from diagnosis to sampling[b]	8.6 [1.0-299.1]	17.7 [3.9-299.1]	7.6 [1.0-110.0]	-	0.113
Renal involvement					
Hypertension[a]	5 (10.6)	3 (27.3) $^{+}$	2 (5.6)	-	**0.041**
Serum creatinine mg/dL[b,c]	44.5 [4-76]	45 [33-76]	36 [4-74]	-	0.085
UACR mmol/mg[b]	0.0 [0.0-2358]	186.8 [94.4-2357.7]$^{+++}$	0.0 [0.0-27.4]	-	**< 0.001**
Urinary creatinine mmol/L[b]	6.8 [0.7-34.3]	3.3 [0.9-15.4]	6.7 [0.7-22.9]	8.7 [3.4-34.3]	0.208
Biopsy proven nephritis[a]	-	7 (63.6)	1 (2.8)	-	-
ISKDC Grade					
II[a]	-	2 (0.9)	-	-	-
IIIa[a]	-	-	1 (2.8)	-	-
IIIb[a]	-	4 (0.36)	-	-	-
IV[a]	-	1 (0.9)	-	-	-
Medications	8 (17.0)	6 (54.5)	2 (5.6)		
Corticosteroids[a]	6 (12.8)	5 (45.5)	1 (2.8)	-	-
ACE inhibitors[a]	3 (6.4)	2 (18.2)	1 (2.8)	-	-
DMARDs[a]	4 (8.5)	3 (27.3)	1 (2.8)	-	-

Table 3.1*: Patients characteristics at baseline. [a]n (%); [b]median [range]. [c]Serum creatinine was available for 22 patients (IgAVN, n=11; IgAVwoN, n=11). UACR: urinary albumin to creatinine ratio. ISKDC: International Study for Kidney Disease in Children Classification. DMARDs: disease modifying anti-rheumatic drugs. DMARDs used in this cohort: hydroxychloroquine, mycophenolate mofetil, azathioprine. Significant p-values are highlighted in bold. $^{+}$ p<0.05 compared to IgAVwoN; *p<0.05 compared to HCs; $^{+++}$ p<0.001 compared to IgAVwoN. Due to rounding, percentages may not add up to 100.*

3.3.2. Urinary IgA and C5a levels in patients with IgAV compared to HCs

The urinary levels of IgA and C5a of IgAV patients were first compared to the HCs group, this is presented in **Figure 3.2**. The urinary IgA/Cr levels were statistically significantly increased in patients with IgAV (69.53 µg/mmol [0.3581-850.1]) compared to HCs (48.33 µg/mmol [14.76-124.7]; *p*=0.026). Similarly, the urinary C5a/Cr concentrations were statistically significantly increased when comparing patients with IgAV (19.74 ng/mmol [4.083-345.1]) to HCs (11.97 ng/mmol [2.499-73.86]; *p*=0.042).

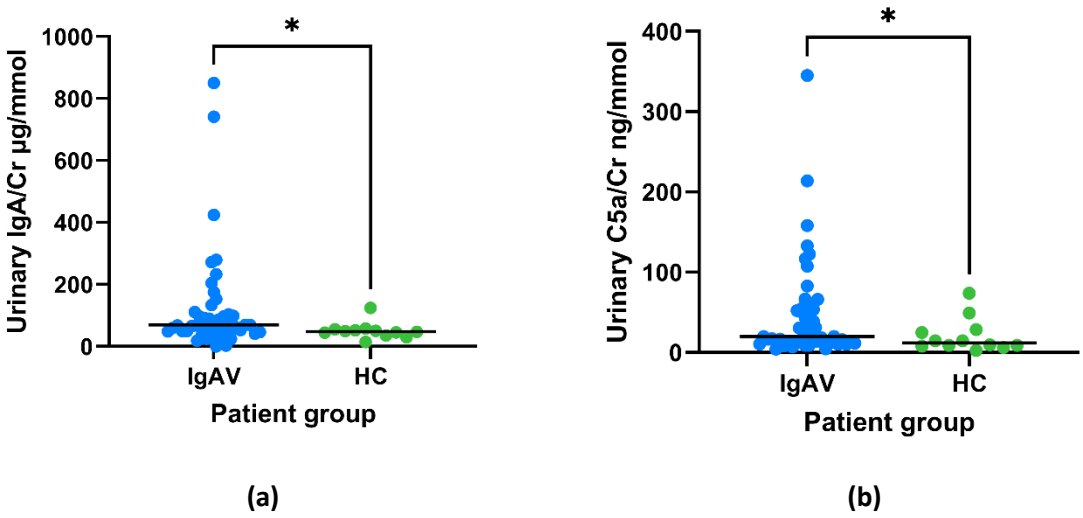


***Figure 3.2**: Urinary IgA/Cr **(a)** and C5a/Cr **(b)** levels between patients with IgAV compared to HCs with median lines. Asterisks indicate a statistically significant difference between groups (* - p < 0.05). Results are normalised to urinary creatinine (Cr).*

3.3.3. Urinary IgA and C5a levels in patients with IgAVN

Patients were then grouped according to the presence of nephritis to identify any difference in the urinary concentrations of IgA and C5a, results are presented in **Figure 3.3**. The urinary IgA/Cr levels were statistically significantly increased in patients with IgAVN (205.0 µg/mmol [51.8-850.1]) compared to IgAVwoN (64.9 µg/mmol [0.4-741.1]; *p*=0.006) and HCs (48.3 µg/mmol [14.8-124.7]; *p* < 0.001). Similarly, the concentrations of urinary C5a/Cr were also statistically significantly elevated in patients with IgAVN (66.4 ng/mmol [12.5-345.1]) compared to both IgAVwoN (17.0 ng/mmol [4.1-122.4]; *p*=0.006) and HCs (12.0 ng/mmol [2.5-73.9]; *p*=0.001). No statistically significant difference was observed between HCs and IgAVwoN. There were clear outliers with extremely increased urinary IgA and C5a levels in both the IgAVN and IgAVwoN groups but not in the HCs.

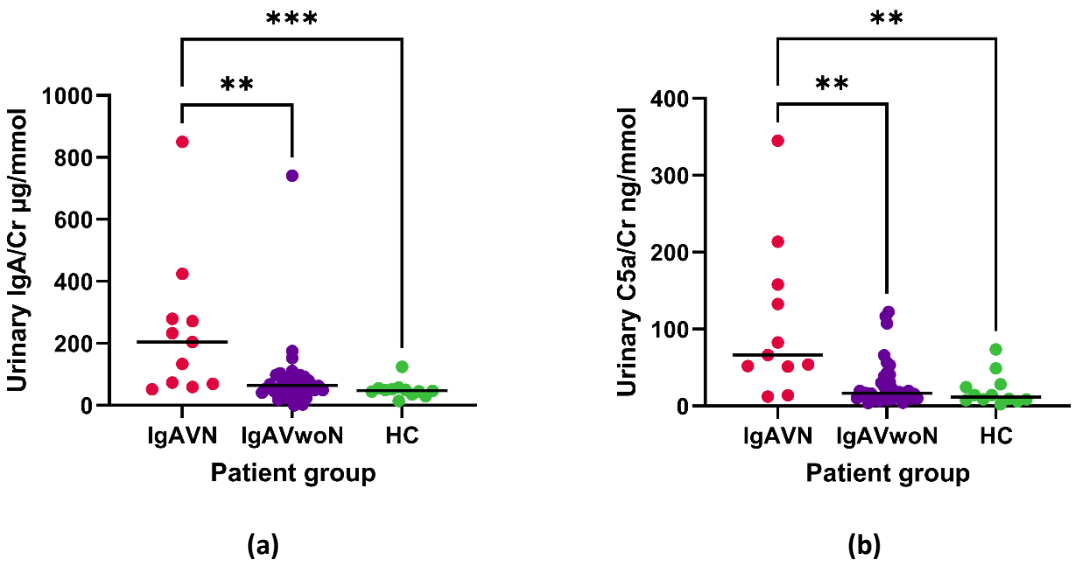


***Figure 3.3**: Urinary IgA/Cr **(a)** and C5a/Cr **(b)** levels by patient group with median lines. Asterisks indicate a statistically significant difference between groups (** - $p < 0.01$; *** - $p < 0.001$). Results are normalised to urinary creatinine (Cr).*

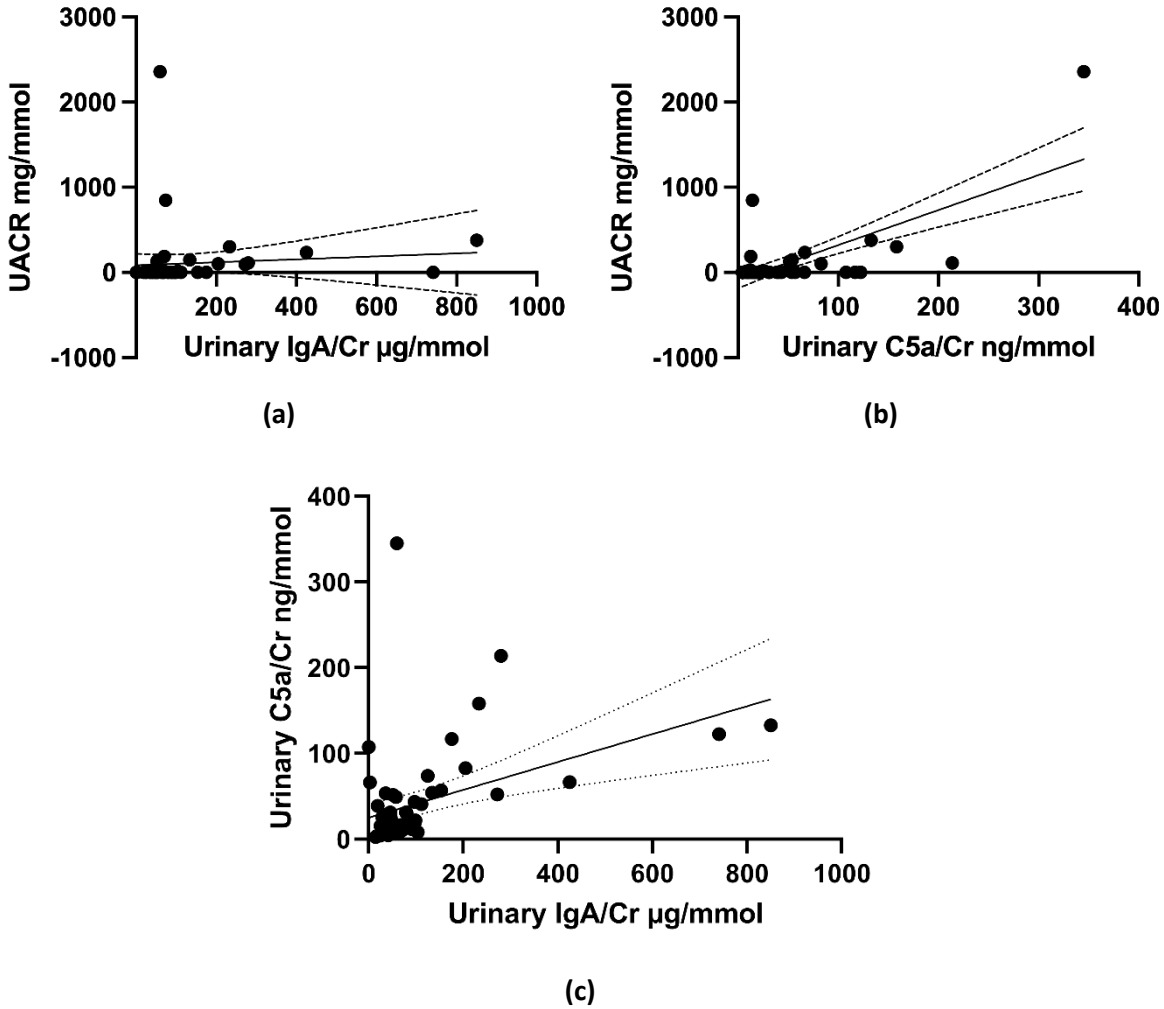


***Figure 3.4**: Correlation between urinary IgA/Cr and UACR in patients with IgAV **(a)**; C5a/Cr and UACR in patients with IgAV **(b);** IgA/Cr and C5a/Cr in the whole cohort **(c)**. The linear regression best fit line is shown with 95% confidence bands.*

3.3.4. Correlation of IgA to C5A and to proteinuria

Urinary C5a concentrations were highly positively correlated to the UACR of patients with IgAV (r=0.7120; *p*=<0.0001) whereas no statistically significant correlation was observed for urinary IgA (r= 0.078; *p*=0.601) (**Figure 3.4.a** and **Figure 3.4.b**). The urinary levels of IgA of the whole cohort demonstrated low positive correlation to urinary C5a (r=0.420; *p*<0.001; **Figure 3.4.c**).

3.3.5. Serum IgA concentrations

In a subgroup of 21 children for which serum samples were available (IgAVN, n=6; IgAVwoN, n=7; HCs, n=8), the IgA serum concentrations were evaluated (**Figure 3.5**). There was a trend towards higher serum IgA levels in children with IgAV (952.7 µg/mL [208.3-2400]) compared to HCs (606.2 µg/mL [473.0-1492]; *p*=0.336) although this was not statistically significant. This was also observed when stratifying patients according to the presence of nephritis but without reaching statistical significance (IgAVN - 1059 µg/mL [208.3-2400]; IgAVwoN - 835.5 µg/mL [284.6-1647]; HCs - 606.2 µg/mL [473.0-1492]; *p*=0.544).

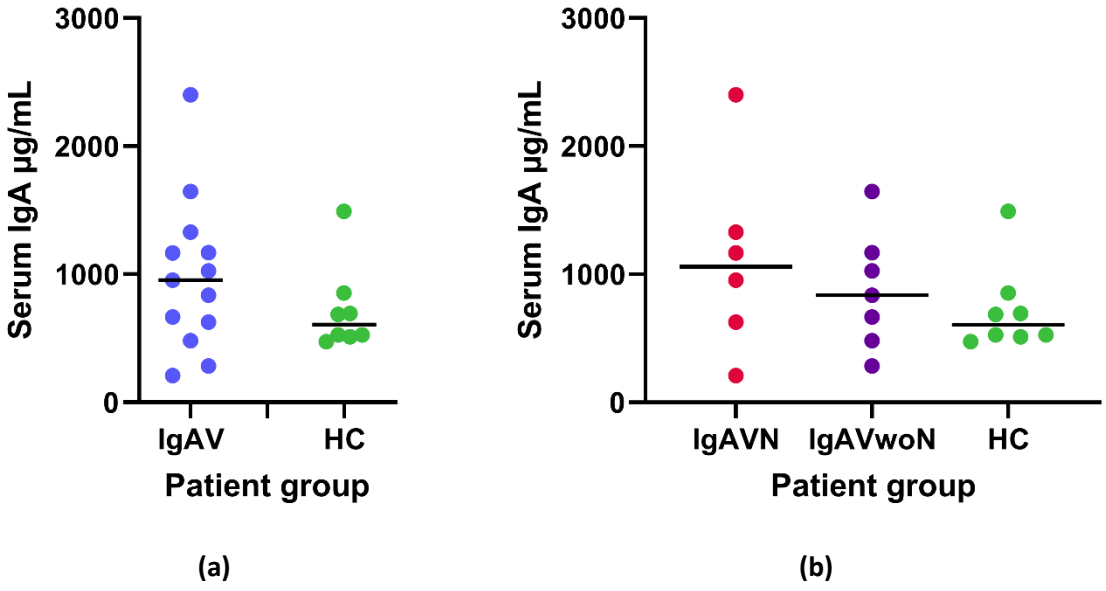


***Figure 3.5**: Serum IgA levels in patients with IgAV compared to HCs **(a)** and in patients with IgAVN, IgAVwoN and HCs **(b)**.*

In addition, the urinary IgA/Cr levels did not correlate to the serum IgA levels in this sub-group (r=0.120; *p*=0.603) (**Figure 3.6**).

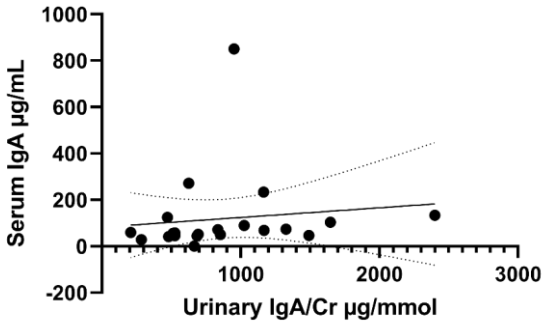


Figure 3.6*: Correlation between urinary IgA/Cr and serum IgA levels with best fit line and 95% confidence bands.*

3.3.6. ROC curves and logistic regression model

Receiver operating characteristic (ROC) curves were generated to assess the ability of IgA and C5a to discriminate between patients with IgAV compared to HCs (**Figure 3.7.a**) and patients with IgAVN compared to IgAVwoN (**Figure 3.7.b**). Urinary IgA was acceptable at distinguishing patients with IgAV from HCs whereas C5a was non-discriminant (IgA – AUC 0.709, 95%CI [0.567-0.852], *p*=0.026; C5a – AUC 0.692, 95% CI [0.521-0.862], *p*=0.042). Using this cross-sectional data, both markers were individually excellent at discriminating patients with and without nephritis (IgA – AUC 0.811, 95%CI [0.664-0.958], *p*=0.002; C5a – AUC 0.826 95% CI [0.676–0.975], *p*=0.001). When combined together, IgA and C5a remained excellent at discriminating IgAVN vs. IgAVwoN with an improved AUC (AUC 0.848; 95% CI [0.708-0.989]; *p*<0.001). The addition of age at diagnosis into the model further improved its diagnostic ability to outstanding (AUC 0.907; 95% CI [0.821-0.992]; p<0.001) (**Figure 3.7.c**).

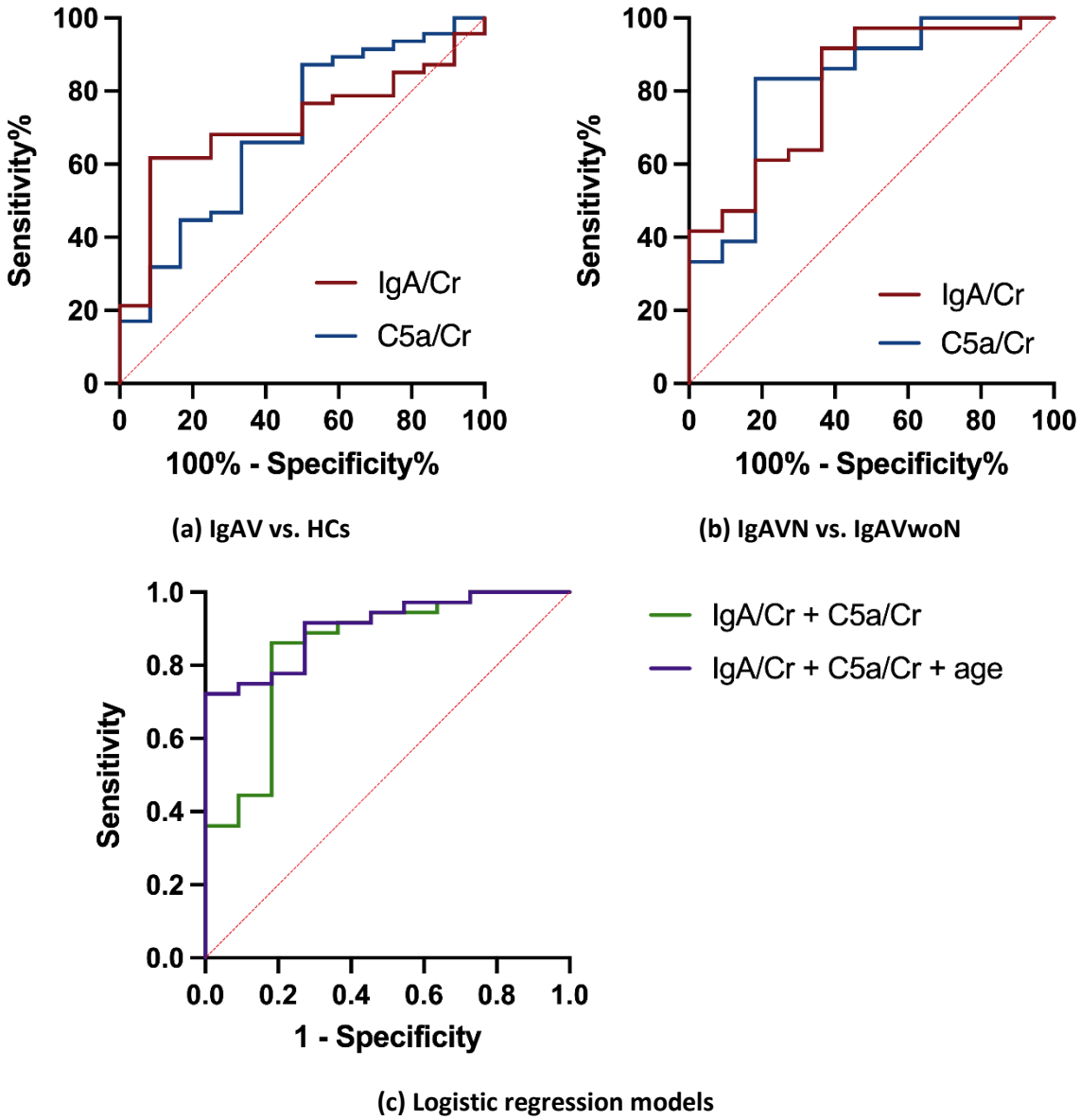


***Figure 3.7**: Receiver operating characteristic (ROC) curve analyses. ROC curves for the ability to discriminate IgAV patients from HCs **(a)** and IgAVN from IgAVwoN **(b)**. ROC curves for the logistic regression models of IgA + C5a (Hosmer-Lemeshow test p=0.302) and IgA + C5a + age (Hosmer-Lemeshow test p= 0.782) **(c)**.*

3.4. Discussion

This chapter aimed to assess the urinary concentrations of IgA and C5a in a cohort of children with IgAV in order to distinguish between patients with and without nephritis. Urinary IgA and C5a levels were statistically significantly increased in IgAV compared to HCs. When grouping patients according to the presence of nephritis, the urinary levels of IgA and C5a were statistically significant between the IgAVN group and both IgAVwoN and HCs, with no statistically significant difference observed in the IgAVwoN vs. HCs comparison. This suggests that urinary IgA and C5a are only increased in the presence of nephritis.

3.4.1. IgA

The finding of elevated urinary IgA/Cr concentrations in patients with IgAVN is expected, since IgAVN is characterised by mesangial deposition of IgA1-containing immune complexes (44). Pillebout and colleagues also reported increased urinary IgA concentrations in children with IgAVN and in this study, urinary IgA/Cr performed better at identifying patients with nephritis (AUC 0.86; 95% CI [0.75–0.96]; p<0.0001) than in the current study. Interestingly, they reported a correlation of IgA/Cr with proteinuria (195), which we did not find. However, it is important to note that the correlation they reported was relatively weak (r=0.47; *p*<0.006) (193, 195). In a prospective cohort of adults with IgAV, urinary IgA levels were good predictors of poor renal outcomes with an optimum urinary IgA/Cr cut-off at 1.13 g/mmol (sensitivity of 76.2%, specificity of 80.0%) (196). Although our assay only assessed the total IgA levels, urinary concentrations of gd-IgA1 and its immune complexes have also been proposed in the literature to be both specific and prognostic markers of IgAVN and IgA nephropathy. In these reports, in children with IgAV the urinary IgA-sCD89 complex levels were increased in patients with both IgAVN and IgAVwoN compared to HCs whereas levels of IgA-IgG complexes were only elevated in the urine of children with IgAVN (195). This was also observed in a Chinese study who reported that the incidence of IgAVN in hospitalised children with IgAV and with a urinary gd-IgA1/Cr ratio ≥ 105.74 was 73% during the six-months follow up (197). Similar findings have been reported for IgA nephropathy, with some specificity of urinary gd-IgA1 to differentiate between IgA nephropathy and other renal diseases (198-200). This reinforces the use of urine as a potential promising biofluid for providing insight into the pathophysiology and monitoring disease activity.

Using a smaller sub-cohort of 21 children, we report no significant difference between the levels of serum total IgA in both IgAV vs. HCs or IgAVN vs. IgAVwoN/HCs. Although circulating IgA levels are not a recognised diagnostic tool in IgAV, some studies have reported elevated serum IgA levels in children with IgAV regardless of the presence of renal involvement (195, 201), but results are conflicting across studies (202, 203). In addition, a meta-analysis concluded that serum IgA levels were not associated with renal involvement (7). However, the IgA/C3 ratio may be of prognostic use to predict renal outcomes and histologic severity in IgAVN (204). In a similar way to the urine, extensive evidence supports that circulating levels of gd-IgA1 levels are elevated in IgAVN patients and this may correlate to disease activity too, although further validation is required (46, 48, 195, 197, 205-208). The lack of correlation in the current chapter between serum and urinary IgA is interesting. A previous study reported that the relative degree of galactose deficiency was higher in urinary IgA1 than in the serum of adult patients with IgA nephropathy, suggesting preferential excretion of gd-IgA1 (198). Further exploration of the O-glycan (and possibly the N-glycan) profiles in both the urine or sera of

children with IgAV, for example through proteomic studies, would allow for better characterisation of the disease and improve our understanding of its pathophysiology.

It remains unclear whether our findings of elevated urinary IgA/Cr concentrations result from glomerular basement membrane permeability due to renal damage or intra-renal/post-renal secretion of IgA. Identifying whether the IgA is under its polymeric secretory (sIgA) or monomeric circulating form would also be useful in answering this question. The healthy urinary tract also produces sIgA but its mechanism of action is unknown (209), and urinary IgA has been reported to be raised in children with acute urinary tract infections (210). In addition, IgA is a large protein (serum IgA – 160 kDa and secretory IgA, sIgA, – 385 kDa) and in comparison, albumin is only 66.5 kDa (211). Current knowledge is that proteins with a molecular weight of <15 kDa are freely filtered through the glomeruli, proteins up to 45 kDa are quite rapidly filtered and proteins between 45 to 60 kDa tend not to be filtered. Plasma proteins larger than 60 kDa are believed to not be filtered through the healthy kidney (212). However, in renal disease, it is feasible that larger molecules could be filtered but the extent to which this is possible remains unknown.

3.4.2. C5a

Although the role of the complement system is well-recognised in both the renal and systemic manifestations of IgAV, as previously described in (**1.1.3.5**), to our knowledge this is the first study to report elevated urinary C5a levels in IgAVN. Histologically, IgAVN is characterised by IgA deposits in the mesangium and C3 co-localisation is commonly seen (19, 213), as confirmed in this patient cohort where histological deposition of C3 was seen in 6/7 (85.7%) of the biopsy-proven IgAVN group. Deposition of the complement components MBL, MBL-associated serine protease (MASP-1), C3b/C3c, C5b-9, and C4 have previously been reported in the renal tissues of patients with IgAVN (55, 56, 189), with C5b-9 and C4d deposits linked to poor renal outcomes (59). In addition, one recent study reported the presence of skin deposits of properdin, MBL, MASP1/3 and MASP 2 in adults with skin-limited IgAV (54).

Hence in IgAV, it is thought that complement activation occurs through the mannose-binding lectin and alternative pathways, and not through the classical pathway (44). Regardless of the route used, the same common pathway is ultimately activated: the binding of C3b to C3 convertase forms C5 convertase, an enzymatic complex which then cleaves C5 into C5a and C5b. C5b contributes to the formation of the membrane-attack-complex (MAC, C5b-9) with complement components C6, C7, C8 and C9 whilst the anaphylatoxin C5a plays a crucial pro-inflammatory role by recruiting inflammatory cells to the site of complement activation. C5a is a potent neutrophil chemoattractant and can bind

to two G-protein coupled receptors, C5aR1 and C5aL2, which stimulates further pro-inflammatory signalling pathways (51).

In vitro, C5a was shown to up-regulate the expression of IL-8 and MCP-1 (57), which urinary levels have been previously reported to be raised in children with IgAVN (128), as well as the endothelial adhesion molecules E-selectin and ICAM-1 (57), both of which have also been linked to the pathogenesis of IgAV by previous studies (214, 215). Higher plasma levels of C3, C5a and Bb but not C4a were reported in the plasma of children in the acute phase of IgAV compared to HCs (57). In adults with IgA nephropathy, patients with >50% crescents demonstrated higher urinary C5a levels and this phenomenon was not seen in plasma (216). The urinary C5a/Cr concentration reported in adult patients with IgA nephropathy correlated with proteinuria in several studies (216-218), which is in keeping with our findings.

To date, there is only one case report of the use of a complement inhibitor in an adult with rapidly progressive glomerulonephritis due to IgAVN (Narsoplimab, a monoclonal antibody against mannan-binding lectin serine protease 2 -MASP-2-), with a successful outcome in avoiding dialysis (219). Therapeutic targeting of the complement system in other forms of glomerulonephritis are evolving. Avacopan, a C5aR1 antagonist, was recently approved in the United-States and Japan for treating adults with ANCA-associated vasculitis (220) and a short-term trial recently showed Avacopan was effective at improving proteinuria in a small cohort of adult patients with IgA nephropathy (221). In addition, local complement activation may be involved in the mechanisms of crescent formation in IgA-mediated glomerulonephritis (222), hence Wang and colleagues proposed that selective complement inhibition may prevent or treat crescentic IgA nephropathy (216). As a result, 12 clinical trials are ongoing for the treatment of IgAN with targets including complement components C3 (ClinicalTrials.gov; NCT02062684), C5 (NCT04564339; NCT03841448), Factor D (NCT05097989; NCT05162066), Factor B (NCT04557462; NCT03373461; NCT04014335; NCT04578834) and MASP-2 (NCT02682407; NCT03608033). However, none of these clinical trials are currently recruiting children.

These findings emphasise the role of the complement system in the pathophysiology of IgAVN and that it may represent a potential treatment target. However, the exact prognostic value of urinary C5a in IgAV remains unknown and further longitudinal studies are needed to investigate its timing of onset and explore its potential use for early clinical intervention.

3.4.3. Limitations

The work described in this chapter has several limitations. First, due to its cross-sectional nature and the heterogenous cohort including variable timing from diagnosis to sampling. Active disease may have not been present in the children with IgAVwoN for whom samples were taken a few

weeks after their first presentation. We were unable to assess the prognostic value of IgA and C5a or any correlation to glomerular crescents, due to the small number of patients. For the urinary IgA ELISA standard curve, linear regression was used as it constituted a better fit for the data, instead of a four-parameter fit logistic regression, which was originally recommended by the manufacturer. The dilution-factor optimisation experiment should have ideally included one sample with IgAVN, one with IgAVwoN and one HCs to find out the best dilution for each sample type, however, the clinical data were not available at this time.

These findings would have benefited from a disease-control cohort to identify any specificity of IgA and C5a to IgAVN compared to other forms of glomerulonephritis (i.e. IgA nephropathy, ANCA-associated vasculitis, lupus nephritis).

3.5. Conclusions

Using a cohort of 59 children including 47 with IgAV, this chapter demonstrated that urinary IgA and C5a were increased in children with IgAVN. These findings highlight the potential utility of urinary IgA and the role of the complement system in this disease that warrants further analysis in larger longitudinal studies.

4. Urinary protein array analysis to identify key inflammatory markers in children with IgA vasculitis nephritis

4.1. Introduction

Nephritis (IgAVN) is the most serious long-term manifestation of IgAV. IgAVN occurs in 40 to 50% of children, and although it is mainly self-limiting, 1–2% of children progress to chronic kidney disease (CKD) stage 5 (11, 19). For this reason, common clinical practice is that all children with IgAV should have at least 6 months of urinalysis testing and blood pressure monitoring following the first presentation (74, 87). The rising presence of proteinuria is suggestive of evolving nephritis, and the gold standard for confirming and grading the diagnosis is to perform a renal biopsy, which remains an invasive procedure (78).

Histologically, IgAVN is characterised by mesangial deposition of pathogenic galactose-deficient IgA1 containing immune complexes, complement activation and neutrophil infiltrates in the glomerulus (44). Yet, the exact pathophysiology of IgAV and the reasons why some patients develop significant renal involvement remains largely unknown. Identifying the variations in urinary proteome profiles between patients with and without nephritis may aid understanding of the pathophysiology of IgAVN and direct the identification of new targets for future validation studies.

4.1.1. Aims

The aim of this chapter was to perform a large exploratory protein array analysis of urinary markers of kidney inflammation and/or injury to provide insight into the pathophysiology of IgAV using a select cohort of children with established IgAVN.

4.2. Materials and methods

4.2.1. Patient selection and definitions

Children were recruited as part of the IgA Vasculitis study. Children of any sex aged <18 years old at diagnosis were eligible to take part. Patients were diagnosed clinically with IgAV according to the EULAR/PRINTO/PReS criteria (223). A minimum follow-up period of 12 months from sample collection was required, and an available urine sample during the follow-up period. Patients were considered to have a normal outcome at 12 months if they were discharged from the 6 month nurse-led HSP pathway (224) and did not have any further IgAV-related presentations to the institution. Exclusion criteria were as follows: (i) diagnosis of IgAV uncertain or in doubt; (ii) other concurrent inflammatory or renal condition; (iii) incomplete follow up; (iv) urine sample obtained at a point greater than 12 months from diagnosis; (v) undergoing dialysis.

The patients with IgAV were subdivided according to the presence of nephritis. Nephritis (termed the "IgAVN" group) was defined as a urinary albumin to creatinine ratio (UACR) > 30 mg/mmol at the time of sampling (223) and a kidney biopsy demonstrating IgAV prior to or within 7 days of sample collection. In order to identify cases with more severe renal inflammation, patients were selected from the IgA Vasculitis study cohort using those patients with the highest UACR. Patients with no renal involvement (termed the "IgAVwoN" group) were patients with a negative urine dipstick for protein and blood at the time of sampling and with an uncomplicated disease course, defined as no further presentation due to IgAV, a UACR < 10 mg/mmol at any point during their follow up and those who received no treatment apart from simple analgesia. A UACR of 0 mg/mmol was assumed for urine dipsticks negative for protein. Hypertension was defined as a systolic blood pressure above the 95th centile for the child's age, sex and height for <16 years old or >140 mmHg for children 16 years and older (190).

Healthy controls (HCs) were recruited to provide a one-off, age and sex-matched clean-catch, midstream spot urine sample. These were children aged <18 years old who were attending for non-inflammatory day case investigations or surgery and with not known relevant medical history or not taking any regular medication.

4.2.2. Data collection

Demographics and clinical data were collected at the time of the sample collection to provide baseline clinical characteristics. This included sex, age, blood pressure, height, serum creatinine (if available), UACR, renal histology report, clinical history and any medication. The primary outcome at 12 months was either discharged or remaining under follow up.

4.2.3. Sample processing

Biological samples were processed in accordance with a standard operating procedure. Healthy control urine samples were tested for bacterial contamination using a urine dipstick and they were discarded if they demonstrated positivity for leukocytes, nitrites, blood or > +1 for protein. Urine samples were centrifuged at 300×g for 10 mins, transferred into a new falcon tube, centrifuged again at 300×g for 10 mins and aliquoted into 1mL sterile Eppendorf tubes for storage. Samples were stored at -80 °C and were thawed at room temperature on the day of the experiment and 300×g for 5 min immediately before use. For this chapter, only samples that had never thawed prior to the experiment were used.

4.2.4. Assays

Human Kidney Biomarker Array Kit and Human XL Cytokine Array Kit (R&D Systems, Minneapolis, MN, USA) were used to assess the relative concentrations of 38 predefined markers of

kidney damage and 105 inflammatory cytokines (total of 124 proteins, 19 were duplicated as they were evaluated in both kits). The full list of the proteins assessed can be found in **Appendix 4**. Both kits were performed according to the manufacturer's instructions. Briefly, each array kit consisted of 4 nitro-cellulose membranes with spotted capture and controls antibodies embedded in duplicates, each membrane allowing for one urine sample to be assessed (therefore four patients per kit could be analysed). Three sets of each kit (12 membranes) were available, which allowed for the analysis of 12 different patients' urine samples. The assays were conducted by performing 4 membranes at a time per day and subjects were intentionally divided to include at least one of each group to reduce selection bias. Due to the semi-quantitative nature of the membranes, we did not assess for intra/inter-variability, but we calculated Pearson's correlation coefficient on the fold changes of the duplicated proteins.

For clarity, the Human Kidney Biomarker Array kit will be further referred to as "Kit K" and the Human XL Cytokine Array kit as "Kit C" throughout the study.

4.2.4.1. *Human Kidney Biomarker Array—Kit K*

The nitrocellulose membranes were blocked for 1 hour using the provided array buffer and incubated with the capture antibody cocktail and 500 µL of thawed urine at 4 °C overnight on a rocking platform shaker. Urine samples were frozen again pending Kit C analysis. Membranes were then washed, incubated with horseradish peroxidase-conjugated Streptavidin (streptavidin-HRP) and chemiluminescence reagents. Signal intensity was measured using enhanced chemiluminescence on the ChemiDoc MP Imaging System (Bio-Rad Laboratories Ltd., Watford, UK).

4.2.4.2. *Human XL Cytokine Array Kit—Kit C*

Membranes were blocked for 1 hour using the provided array buffer and incubated with 400–450 µL of urine (previously thawed) at 4 °C overnight on a rocking platform shaker. Membranes where then washed, incubated with biotinylated detection antibodies, followed by the addition of Streptavidin-HRP and chemiluminescence reagents. Signal intensity was measured using enhanced chemiluminescence on the ChemiDoc MP Imaging System (Bio-Rad Laboratories Ltd., Watford, UK).

4.2.5. Creatinine quantification

All results were corrected for the urinary creatinine concentrations. Automated quantification of urinary creatinine was performed by the Biochemistry Department (Alder Hey Children's NHS Foundation Trust, Liverpool, UK) using a previously described enzymatic creatinine method (191) on the Abbott Architect Ci8200 (Abbott, Illinois, USA).

4.2.6. Ethical approval

All procedures involving human subjects were conducted in accordance with NIHR Good Clinical Practice, HTA Codes of Practice, the Declaration of Helsinki and comparable ethical standards. This study was part of the IgA Vasculitis study which was approved by HRA and Health and Care Research Wales (HCRW) on June 21, 2019 (REC 17/NE/0390, protocol UoL001347, IRAS 236599). Written informed consent was obtained from parents and children prior to any study-related procedure.

4.2.7. Data analysis

The intensity of each individual chemiluminescence signal was measured using ImageJ software (NIH) (225) and ImageJ Protein Array Analyser Macro (226) with automatic background removal. To determine the relative concentration of each protein, the average value of the duplicates was measured and normalised to the average of the 6 positive control points, the volume of urine aliquot used and the urinary creatinine concentration. For each protein, fold change was obtained by calculating the ratios of the average relative concentration per group for IgAVN/IgAVwoN, IgAVN/HCs and IgAVwoN/HCs. Data are presented as the fold change (FCx) between two groups.

4.2.8. Statistical analysis

Clinical and demographical data were compared with the Statistical Package for the Social Science (SPSS) version 27.0 software for Windows (IBM Corp, Armonk, NY, USA). Due to the small sample size, data were assumed to be non-normally distributed and the Mann–Whitney U/Kruskal–Wallis with Dunn–Bonferroni post hoc tests were used for continuous variables. The Pearson's chi-square was applied to categorical variables. For the analytes, multivariate principal component (PC) analysis was performed using the MetaboAnalyst 5.0 online platform (227) with log2 transformation and pareto scaling. The Student's *t*-test was applied on the log2 transformed data. *p*-values and fold change were combined using the "EnhancedVolcano" package (228) in R Statistical Software version 4.1.2 (R Foundation for Statistical Computing, Vienna, Austria) to generate volcano plots and identify significant proteins. Linear correlation was assessed with the Pearson's correlation coefficient: a coefficient of < 0.30 was considered negligible, 0.30-0.50 low correlation, 0.50-0.70 moderate correlation, 0.70-0.90 high correlation and 0.90-1.00 very high correlation (193). A *p*-value of <0.05 was considered statistically significant. Protein concentrations were considered to be elevated for FC ≥ 2.0 (2x) and reduced for FC ≤ 0.5 (0.5x).

4.3. Results

4.3.1. Paediatric cohort

The study identified 8 children with IgAV from a cohort of 51 recruits to be included in this exploratory analysis. This included four children with nephritis selected as detailed previously and four children without any evidence of past or recent nephritis (termed as 4 IgAVN and 4 IgAVwoN). Four HCs were randomly selected from a cohort of 18 HCs children who had been previously recruited to the IgA Vasculitis study. Demographics and baseline characteristics are presented in **Table 4.1**. The male:female ratio was 1:1, and the median age was 7.6 years old (range: 4.0–13.4). In those patients with IgAVN, renal involvement was confirmed histologically in all patients (ISKDC grade II, n = 1; grade IIIb, n = 3), and the median UACR at the time of sample collection was 542.2 (110.4–2357.7) mg/mmol. All patients within the IgAVN cohort had nephrotic range proteinuria as an indication for conducting a renal biopsy with a mildly reduced serum albumin concentration but no evidence of peripheral oedema. They all had normal serum complement titres and normal renal function; two had high blood pressure. The histological analysis demonstrated diffuse proliferative glomerulonephritis with <50% crescents in three patients and focal proliferative glomerulonephritis in one patient. All patients had IgA-positive staining on immunofluorescence (IF) and three out of the four patients had C3 positivity on IF. One patient had patchy evidence of acute tubular inflammation and one patient had <5% interstitial fibrosis. Regarding treatment at the time of sample collection, one IgAVN patient was taking oral prednisolone and one patient was taking both prednisolone and azathioprine. Subsequent treatments included azathioprine and prednisolone for one patient; mycophenolate mofetil, ACE inhibitors and prednisolone for another; ACE inhibitor only for the third patient; prednisolone and azathioprine for the fourth patient. One healthy control was prescribed somatotropin at the time of sample collection. None of the IgAVwoN patients received any medication. At 12 months, all of the IgAVwoN patients were discharged and three patients with IgAVN remained under follow up.

	Overall	IgAVN	IgAVwoN	HCs	*p*-value
n	12	4	4	4	-
Male/female	6/6	2/2	2/2	2/2	1.000
Age, years [a]	7.6 [4.0–13.4]	9.9 [5.2–12.2]	5.1 [4.0–6.8]	8.8 [7.5–13.4]	0.058
Time from diagnosis to sample (weeks) [a]	14.8 [1.0–32.0]	14.8 [5.3–25.3]	19.2 [1.0–32.0]	-	0.773
Renal involvement					
Hypertension [b]	2	2	0	-	0.102
Serum creatinine, mg/dL [a]	42.0 [32.0–54.0]	43.5 [33.0–54.0]	32.0 [32.0–32.0] [c]	-	0.400
Urinary creatinine, mmol/L [a]	7.8 [1.2–19.9]	6.6 [1.6–13.2]	7.8 [1.2–9.4] ± 3.6	9.3 [6.8–19.9]	0.668
UACR, mg/mmol [a,d]	0.0 [0.0–2357.7]	542.2 [110.4–2357.7]	0.0 [0.0–0.0]	0.0 [0.0–0.0]	-
Biopsy proven nephritis[a]	-	4	0	-	-
ISKDC Grade					
II [b]	-	1	-	-	-
IIIb [b]	-	3	-	-	-
Medications [b]	3	2	0	1	-
Corticosteroids [b]	2	Prednisolone (2)	-	-	-
DMARDs [b]	1	Azathioprine (1)	-	-	-
Other[b]	1	-	-	Somatropin (1)	-
Follow up at 12 months					
Discharged	5	1	4	-	
Still under follow up	3	3	-	-	

Table 4.1*: Patients characteristics at baseline. [a]Median [range]; [b]n; [c]serum creatinine only available for one IgAVwoN patient; [d]UACR of 0 mg/mmol assumed if urine dipstick negative for protein. UACR: urinary-albumin-to-creatinine ratio; ISKDC: International Study for Kidney Disease in Children Classification; DMARDs: disease modifying antirheumatic drugs.*

4.3.2. Exploratory data analysis

Principal component analysis was conducted to visualise the data sets using the first two principal components (PC), and the score plots for each kit are presented in **Figure 4.1** (explained variance of Kit K: PC1 × PC2 = 82.8%; Kit C: PC1 × PC2 = 82.7%). The models demonstrated clear separation between IgAVN and both the IgAVwoN and HCs groups. The IgAVN and IgAVwoN clusters demonstrated some areas of overlap in Kit C.

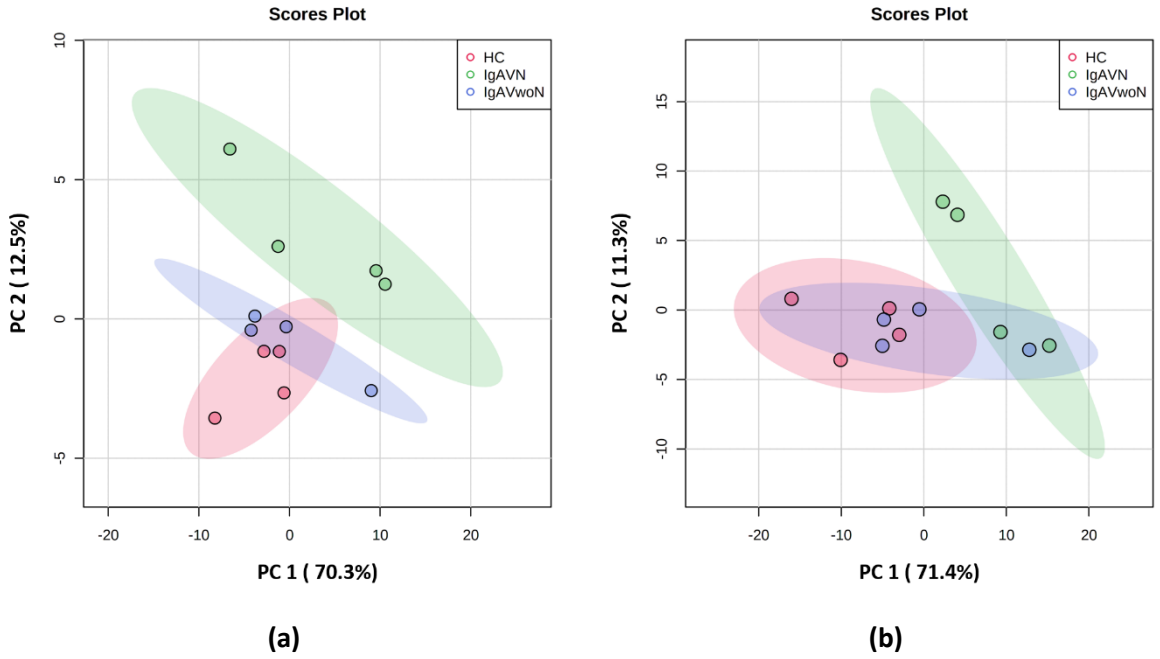
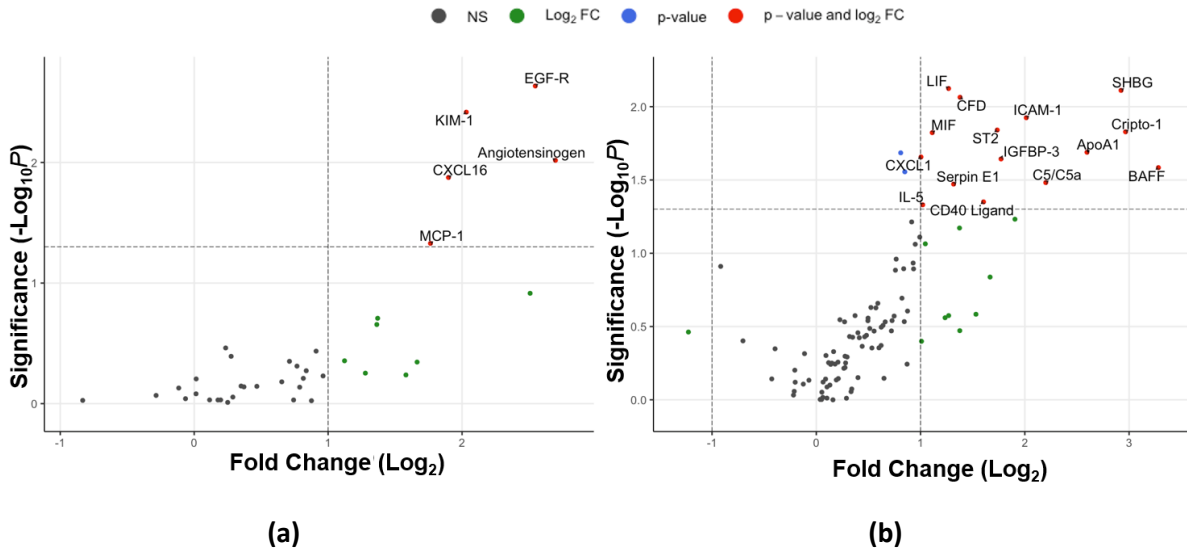


***Figure 4.1**: Exploratory data analysis representation: **(a)** Kit K: —scores plot between principal component (PC) 1 and PC2 with the explained variance shown in brackets; **(b)** Kit C: —scores plot between PC1 and PC2 with the explained variance shown in brackets.*

4.3.3. Comparison between IgAVN and IgAVwoN

Urinary levels of 20 proteins (5 in Kit K -3.1%- and 15 in Kit C -14.3%-) were significantly increased in IgAVN compared to IgAVwoN (**Figure 4.2**). All proteins with a FC ≥ 2.0 are shown in **Table 4.2**, full results can be found in **Appendix 5** and **Appendix 6**. The main features identified were B-cell activating factor (BAFF; 9.7x), Cripto-1 (7.8x), sex-hormone-binding globulin (SHBG; 7.6x), angiotensinogen (AGT; 6.5x), apolipoprotein A1 (ApoA1; 6.0x) and epithelial growth factor receptor (EGF-R; 5.8x). One interleukin (i.e., IL-5) and two components of the complement system (i.e., C5/C5a and complement factor D- CFD-) were also significant, with a fold change of, respectively, 2.0, 4.6 and 2.6. Endoglin (1.75x; p=0.021) and human growth factor (HGF; 1.80x; p=0.028) were both statistically significant but did not reach the fold-change criteria. The urinary concentration of one marker was reduced, but this was not statistically significant (myeloperoxidase; 0.4x; p=0.345).



(a) **(b)**

***Figure 4.2**: Volcano plots of IgAVN vs. IgAVwoN: (a) Kit K; (b) Kit C. The horizontal line represents the statistical significance cut off (−log10(0.05)). Vertical lines represent the fold-change cut-off (log2(0.5)−log2(2.0)).*

Protein	Fold-change	*p*-value
BAFF	9.7	0.026
Cripto-1	7.8	0.015
SHBG	7.6	0.008
Angiotensinogen	6.5	0.010
ApoA1	6.0	0.021
EGF-R	5.8	0.002
IL-1RA	5.7	0.122
C5/C5a	4.6	0.033
KIM-1	4.1	0.004
ICAM-1	4.0	0.012
ENA-78	3.7	0.059
CXCL16	3.7	0.013
IGFBP-3	3.4	0.023
MCP-1	3.4	0.047
ST2	3.3	0.014
DPPIV	3.2	0.145
Annexin V	3.2	0.452
CD40 Ligand	3.0	0.045
Adiponectin	3.0	0.578
PF4	2.9	0.261
CFD	2.6	0.009
Vitamin D BP	2.6	0.337
GH	2.6	0.067
IL-6	2.6	0.196
VCAM-1	2.6	0.221
Serpin E1	2.5	0.034
TWEAK	2.4	0.559
CRP	2.4	0.266
LIF	2.4	0.008
VCAM-1	2.4	0.275
L-FABP	2.2	0.441
MIF	2.2	0.015
Fas Ligand	2.1	0.086
IL-5	2.0	0.047
CD14	2.0	0.399
CXCL1	2.0	0.022
Myeloperoxidase	0.4	0.345

***Table 4.2**: Proteins with a fold-change of at least 2.0 (or ≤0.5) between IgAVN and IgAVwoN identified by both kits, as sorted in order of descending fold change. Bold text represents duplicated proteins that were measured in both kits where results from Kit C are reported.*

4.3.4. Comparison between IgAVN and the HCs

Analysis of IgAVN versus the HCs revealed 68 proteins with significantly increased urinary concentrations (**Figure 4.3** and **Table 4.3**; full results in **Appendix 5** and **Appendix 6**). The highest reported fold change was BAFF (26.5x) followed by angiotensinogen (22.6x), SHBG (13.3x) and MCP-1 (12.0x). Eighteen interleukins out of the 26 assessed by the arrays were significantly elevated compared to the control levels. All of the significant proteins identified by the IgAVN vs. IgAVwoN comparison were also significant in this comparison. No proteins had reduced levels between the two groups.

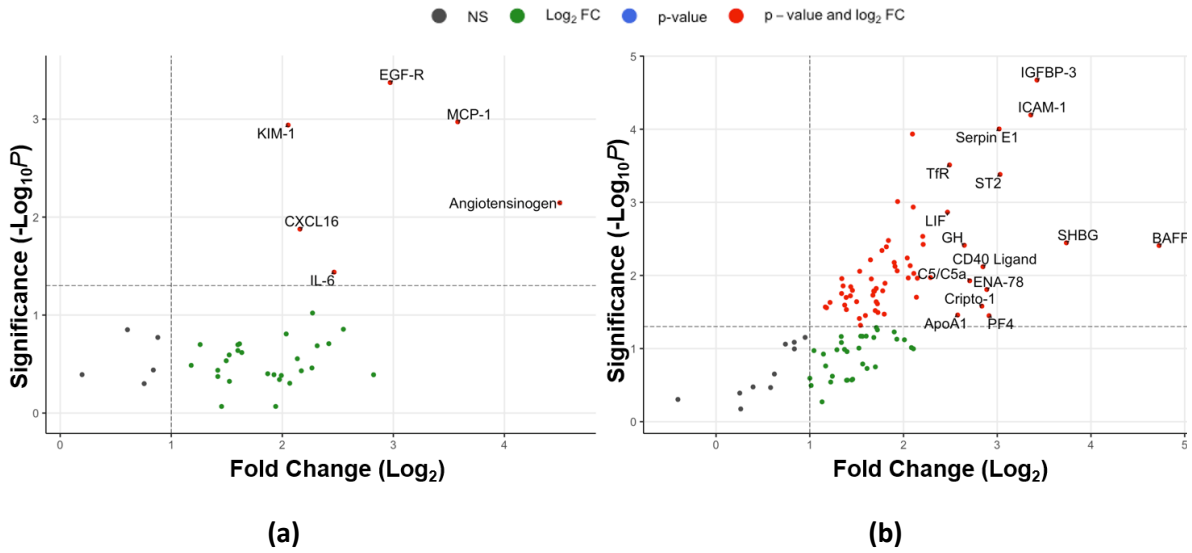


***Figure 4.3**: Volcano plots of IgAVN vs. HCs: **(a)** Kit K; **(b)** Kit C. The horizontal line represents the statistical significance cut off (−log10(0.05)). The vertical line represents the fold-change cut-off (log2(2.0)). Fifteen top hits in term of FC are labelled in (b).*

Protein	Fold-change	*p*-value
BAFF	26.5	0.004
Angiotensinogen	22.6	0.007
SHBG	13.3	0.004
MCP-1	12.0	0.001
IGFBP-3	10.7	< 0.001
ICAM-1	10.2	< 0.001
ST2	8.2	< 0.001
Serpin E1	8.1	< 0.001
EGF-R	7.8	< 0.001
PF4	7.5	0.036
ENA-78	7.4	0.016
CD40 Ligand	7.2	0.008
Cripto-1	7.1	0.026
C5/C5a	6.5	0.012
GH	6.3	0.004
ApoA1	6.0	0.035
TfR	5.6	< 0.001
LIF	5.5	0.001
IL-6	5.5	0.036
IGFBP-2	4.9	0.011
IL-4	4.6	0.004
IL-5	4.6	0.003
CXCL16	4.5	0.013
RANTES	4.4	0.011
IL-22	4.4	0.020
Leptin	4.3	0.009
HGF	4.3	0.001
CXCL1	4.3	< 0.001
PDGF-AA/BB	4.2	0.007
KIM-1	4.1	0.001
TARC	4.1	0.011
MCP-1	4.1	0.006
MIG	3.8	0.001
IL-19	3.8	0.009
Fas Ligand	3.8	0.008
IL-33	3.7	0.007
G-CSF	3.6	0.003
IL-32	3.5	0.004
MIP-3b	3.5	0.013
Angiopoietin-1	3.5	0.034
IL-13	3.4	0.016
CFD	3.4	0.005
IL-10	3.3	0.032
IL-23	3.3	0.024
IL-31	3.3	0.015
IL-27	3.3	0.023
IFN-γ	3.2	0.030
GM-CSF	3.2	0.016
MCP-3	3.2	0.016
IL-24	3.2	0.019
IL-15	3.2	0.011
MIF	3.1	0.006
Pentraxin-3	3.0	0.035
Relaxin-2	2.9	0.048
I-TAC	2.9	0.009
IL-8	2.9	0.039
IP-10	2.8	0.023
IL-16	2.7	0.016
Endoglin	2.7	0.014
Flt-3 ligand	2.7	0.019
Thrombospondin-1	2.6	0.029
BDNF	2.6	0.020
IL-34	2.6	0.025
MIP-1α/MIP-1β	2.6	0.014
Dkk-1	2.5	0.011
MIP-3α	2.5	0.018
TNF-α	2.3	0.023
TGF-α	2.3	0.028
PDGF-AA	2.2	0.027

***Table 4.3**: Significant urinary proteins identified between IgAVN and HCs sorted by fold-change. Bold text represents duplicated proteins that were measured in both kits where results from Kit C are reported.*

4.3.5. Comparison between IgAVwoN and the HCs

The concentrations of 9 proteins were increased in the urine of IgAVwoN patients when compared to HCs (**Figure 4.4**; **Appendix 5** and **Appendix 6**). MCP-1 levels were raised (3.5x; p=0.001) and angiotensinogen displayed a *p*-value close to the significance cut-off (3.5x; *p*=0.055). Urinary IL-13 (2.8x; *p*=0.049), leptin (2.6x; *p*=0.022), IL-19 (2.5x; *p*=0.029), GH (2.4x; *p*=0.017), HGF (2.4x; *p*=0.020), IL-4 (2.3x; *p*=0.036), IL-5 (2.3x; *p*=0.016) and CXCL1 (2.1x; *p*=0.017) concentrations were all elevated in IgAVwoN patients compared to HCs.

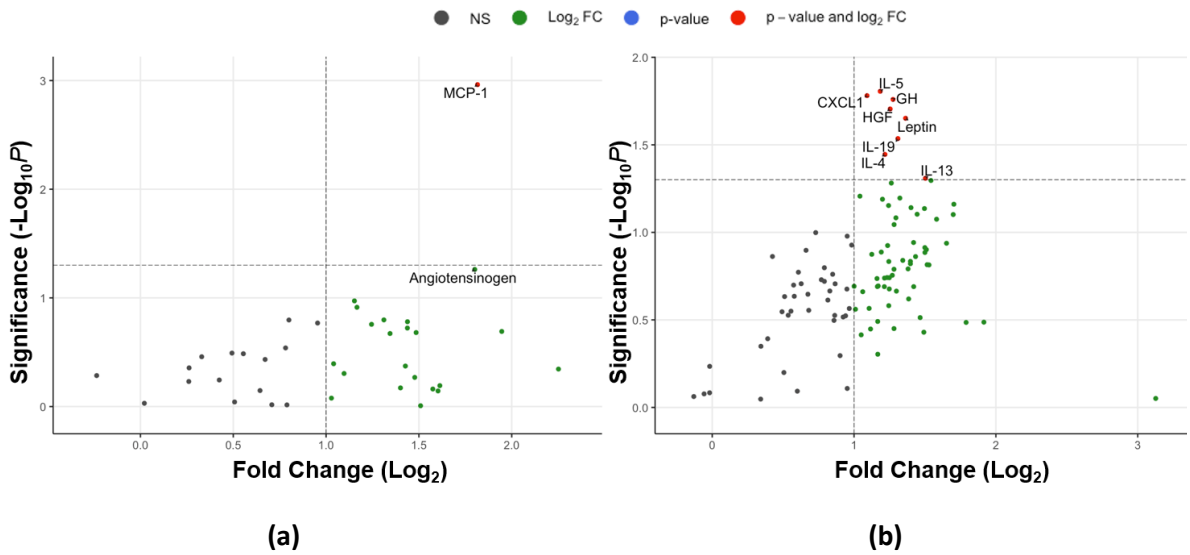


***Figure 4.4**: Volcano plots of IgAVwoN vs HCs. (a) Kit K; (b) Kit C. The horizontal line represents the statistical significance cut-off (-log10(0.05)). The vertical line represents the fold-change cut-off (log2(2.0)).*

4.3.6. Correlation between the Kit K and C

A total of 19 proteins were assessed in both Kit K and Kit C. Moderate positive correlation (r=0.640; $p < 0.0001$) was found between the two kits when comparing the reported average fold changes. The fold changes and *p*-values of six proteins that were reported as statistically significant in at least one kit are reported in **Table 4.4**. There was agreement on a further 13 proteins which did not reach statistical significance in both kits.

Protein	IgAVN vs. IgAVwoN		IgAVN vs. HCs		IgAVwoN vs. HCs	
	Kit K	Kit C	Kit K	Kit C	Kit K	Kit C
CXCL1	**1.8 (0.534)**	**2.0 (0.022)**	**5.0 (0.206)**	**4.3 (<0.001)**	**2.8 (0.539)**	**2.1 (0.017)**
IL-6	2.6 (0.196)	1.8 (0.293)	5.5 (0.036)	3.3 (0.052)	2.1 (0.496)	1.8 (0.197)
IL-10	1.6 (0.659)	1.7 (0.288)	**4.8 (0.346)**	**3.3 (0.032)**	3.1 (0.642)	2.0 (0.203)
MCP-1	3.4 (0.047)	1.9 (0.061)	12.0 (0.001)	4.1 (0.006)	**3.5 (0.001)**	**2.2 (0.134)**
Thrombospondin-1	1.4 (0.719)	0.9 (0.781)	**3.7 (0.397)**	**2.6 (0.029)**	2.6 (0.673)	2.9 (0.153)
TNF-α	1.6 (0.446)	1.1 (0.469)	**2.7 (0.366)**	**2.3 (0.023)**	1.6 (0.963)	2.1 (0.385)

***Table 4.4**: Proteins reported significant by one comparison in at least one kit. Data are presented as the fold change (p-value). The bold text highlights discrepancies in the results of significance between the kits.*

4.4. Discussion

This study aimed to assess the relative concentrations of 124 known markers of inflammation and renal damage in the urine of children with IgAV to identify differences between children with and without nephritis. We report increased urinary concentration of 20 proteins in IgAVN when compared to IgAVwoN. To our knowledge, this exploratory study is the first to evaluate such an extensive panel of urinary proteins in children with IgAVN. In addition, we report some novel findings that provide new insight into the pathophysiology of IgAVN. This discussion will first focus on the urinary proteins with the most increased fold-change and the most promising in terms of therapeutic target, then it will overview the remaining ones identified in the IgAVN vs. IgAVwoN comparison.

4.4.1. B-cell activating factor (BAFF)

The protein with the greatest increased fold-change when comparing IgAVN to IgAVwoN was B-cell activating factor (BAFF). Gd-IgA1 and glycan-specific IgG and IgA antibodies, which are found at increased circulating levels in IgAVN (44), are mainly derived from activated B-cell lymphocytes and effective B-cell maturation relies upon BAFF (229). In our study, BAFF levels were increased by 9.7-fold between IgAVN and IgAVwoN and 26.5-fold between IgAVN and HCs. The role of BAFF in IgAV remains unclear, it has only been explored by one study which did not find any association between BAFF gene polymorphisms and IgAV (230). BAFF is a cytokine of the tumour necrosis factor (TNF) family and some evidence suggests it could activate the tumour necrosis receptor-associated factor 6 (TRAF6)/NF-kB pathway in glomerular mesangial cells consequently contributing to IgA nephropathy pathogenesis (231). BAFF serum levels were reported to be significantly elevated in patients with IgA nephropathy and to correlate with clinical and histological features, suggesting that serum BAFF may be a biomarker of disease severity (231-234). However, Vincent and colleagues were unable to detect urinary BAFF in IgA nephropathy patients, but this study used ELISA analysis in an older population (mean age 44.5 years range [21-69]) (235). In mice, overexpression of BAFF resulted in an IgA nephropathy-renal phenotype and hyper-IgA syndrome (236, 237). In addition to IgA nephropathy,

raised serum BAFF levels have also been linked to various autoimmune diseases such as rheumatoid arthritis (RA), Sjogren syndrome, systemic lupus erythematosus (SLE) and multiple sclerosis (229). Increased urinary BAFF levels and its usefulness as a biomarker has been reported for lupus nephritis (235, 238). BAFF is mainly produced by antigen-presenting cells, including monocytes, dendritic cells, neutrophils, B cells and T cells (239). However, the site of BAFF production in states of nephritis remains unknown, although BAFF was expression in the renal tubular epithelial cells of patients with renal allograft rejection has been reported by a previous study (240, 241).

Thus, targeting B-cell modulation through BAFF inhibition (or through inhibition of a-proliferation-inducing ligand -APRIL- which has a very similar function and structure to BAFF (232)) represents a promising therapeutic approach and may reduce the production of gd-IgA1 and glycan-specific immunoglobulins. In adult patients with SLE, results from clinical trials of blisibimod (a monoclonal antibody against BAFF) reported it was safe and associated with reduced proteinuria and successful biomarkers response (242, 243). In IgA nephropathy, results are awaited for the BRIGHT-SC trial (ClinicalTrials.gov; NCT02062684), a phase II/III study assessing the safety and efficacy of blisibimod in patients with IgA nephropathy, and the interim results reported a significant decrease in B-cells subsets and serum immunoglobulins levels as well as reduction in proteinuria in the blisibimod group (232). The efficacy and safety of another BAFF inhibitor, belimumab, is currently being assessed in a phase II study recruiting adult patients with IgA nephropathy (clinicaltrialsregister.eu; 2017-004366-10). In addition, the preliminary results of a safety and efficacy phase II study of APRIL inhibitor atacicept (ClinicalTrials.gov; NCT02808429) reported that at 24 weeks, a dose-dependent reduction in serum immunoglobulins, including in gd-IgA1, and proteinuria was observed (244).

Our data suggests that BAFF could be involved in the pathophysiology of IgAVN and it may be a promising marker of disease activity as well as a potential drug target. Further analysis is warranted as more evidence supporting the role of BAFF in IgAVN could lead to clinical trials with drugs that have already shown to be safe in adult populations.

4.4.2. Cripto1

One of the other significantly elevated protein in this study was Cripto-1, an embryonic protein that is re-expressed during inflammation, wound healing and tumorigenesis (245). Several *in vitro* studies have shown that Cripto-1 modulates macrophage cytokine secretion resulting in elevated concentrations of pro-inflammatory cytokines TNF-α, IL-6, IL-1β and anti-inflammatory cytokine IL-10 (245, 246), all of which have also been linked to the pathogenesis of IgAV and IgAVN (47). In addition, overexpression of cripto-1 was reported in Crohn's disease, ulcerative colitis and RA pathological

tissues (245) suggesting a link to autoinflammatory processes. Increased urinary concentrations of Cripto-1 in our IgAVN cohort (7.8x) supports a potential role of this modulating protein in IgAVN.

4.4.3. Sex-hormone binding globulin

The potential biological significance of our finding of increased urinary sex-hormone binding globulin (SHBG) levels in IgAVN is unclear. SHBG is a glycoprotein primarily synthesised by the liver and it is involved in transporting androgens and oestrogens. SHBG serum levels are stable in childhood and decrease at puberty, especially in boys (247). *In vitro*, SHBG suppresses inflammation in macrophages (248) and it is downregulated by TNF-α (249) and IL-1β (250). Plus, low plasma SHBG were reported in patients with RA (251) and TNF- α inhibition resulted in an increase in blood levels of SHBG in patients with RA (252) and psoriatic arthritis (253). In our cohort, two of the IgAVN patients were taking prednisolone and the pubertal status of our cohort was not documented. The impact of synthetic glucocorticoids on urinary SHBH is unknown, although some studies have reported lowered SHBG serum levels for patients undergoing treatment with corticosteroids (254).

4.4.4. Angiotensinogen

The significantly increased concentration of urinary angiotensinogen in this study is not surprising because extensive evidence supports the existence of an intrarenal RAAS system which contributes to kidney disease progression (255), and the benefits of RAAS inhibition as a renoprotective treatment is well recognized for glomerular diseases (256). Furthermore, the RAAS system may have a direct role in complement pathway activation through renin-mediated C3 cleavage as demonstrated *in vitro* and suggested through a study evaluating the efficacy of direct renin-inhibition (aliskiren) in dense deposit disease where reduced systemic and renal complement activation was observed (257). ACE gene polymorphisms have also been associated with IgAV susceptibility (258). The majority of the circulating AGT is produced by the liver but local renal production by the proximal tubules has been demonstrated in animal models (259). In a study by Nishiyama and colleagues, urinary AGT (UAGT) levels were raised in patients with IgA nephropathy and this was shown to correlate with renal tissue gene expression of AGT and angiotensin II immunoreactivity. Thus, UAGT was proposed to provide a specific index of intrarenal RAAS activity in patients with IgA nephropathy (260). We found that urinary UAGT concentrations were increased by 6.5-fold in IgAVN compared to IgAVwoN and this was observed by two previous studies that found remarkably elevated UAGT levels in children with IgAVN (261, 262). In addition, a recent systematic review identified UAGT as one of the most promising pre-clinical biomarkers in children with IgAVN. However, urinary renin levels were not significantly different across the groups in the current study. Interestingly in our cohort, there was a trend towards increased UAGT levels in patients without nephritis although this finding was not statistically significant (p=0.055).

4.4.5. Apolipoprotein A1

We report elevated levels of urinary apolipoprotein A1 (ApoA1), which is a novel finding in IgAVN. ApoA1 is the main protein component of high-density lipoproteins (HDL) (263) and although HDL have a protective role in cardiovascular disease and it has been widely studied, more recent evidence suggests HDL may contribute to kidney disease (264) and auto-immunity (265). The kidneys are involved in HDL metabolism and interactions between renal parenchymal cells and potentially harmful lipoproteins could be a factor contributing to CKD initiation / progression (264). A recent study reported elevated urinary ApoA1 in children with various kidney diseases (including 18 children with glomerulonephritis) (266) and multiple adult studies report similar findings (267, 268). Of note, ApoA1 is normally reabsorbed by the renal tubules through cubulin/megalin complexes (269) and urinary ApoA1 levels were particularly elevated in children with diseases involving the proximal tubules (266). Abnormal forms of urinary ApoA1 have been reported in several kidney diseases and an association was found between high molecular weight forms of ApoA1 and relapsing focal segmental sclerosis following kidney transplant (267, 270).

We report increased urinary ApoA1 (6.0x) in nephritis due to IgAV. Identifying whether ApoA1 is excreted in a modified form and its effect in the downstream lipid pathways could provide more information on the renal handling of lipoproteins and their role in this disease.

4.4.6. Complement activation

Activation of both the alternative and mannose-binding lectin complement pathways have been demonstrated in IgAV (44) with skin and mesangial deposits reported to contain complement components C3 and C5-9 (57, 271). In addition, C4d and C5b-9 positivity in renal biopsies has been associated with poor renal outcomes in patients with IgAVN and IgA nephropathy (59). The findings of increased C5/C5a and complement factor D concentrations in the urine of children with IgAVN compared to IgAVwoN (respectively 4.6x and 2.6x) raises the potential role of the complement system in the pathophysiology of this condition and supports this pathway as a potential treatment target. C5 cleaves into C5a, a potent neutrophil chemoattractant, and C5b, which ultimately leads to the formation of the membrane attack complex (MAC) (272). C5a has also been shown to increase production of pro-inflammatory cytokines and endothelial adhesions molecules involved in IgAV pathogenesis by endothelial cells *in vitro* (57) , as discussed in **1.1.3.5**.

Although the alternative complement pathway is continuously active at low level due to spontaneous C3 hydrolysis, cleavage of C3-bound factor B by factor D is essential to form the $C3(H_2O)Bb$ convertase. In addition, factor D also plays an essential role in controlling complement activation through the amplification loop. Hence, its concentration is the limiting factor of the

alternative pathway, as it is ultimately responsible for the activity and output of this route, making factor D a strategic therapeutic target (53). Impaired regulation of the alternative pathway may contribute to the renal phenotype in IgA-mediated glomerulonephritis, as suggested by several studies which showed that genetic variants of factor H (a major negative regulator of the alternative pathway) and factor H-related proteins predispose patients to IgA nephropathy (273-277) or play a role in determining the renal phenotype of patients with IgAV (58). In the current study, the presence of factor D in the urine of patients with IgAVN suggests that activation of the alternative pathway is involved in IgAVN.

4.4.7. Other markers

A further 10 novel urinary proteins were significantly increased in IgAVN, their biological function and potential relevance to the pathophysiology of IgAVN are summarised in **Table 4.5**.

Protein	Biological function and potential pathophysiological role
EGF-R	- Membrane tyrosine kinase receptor, can be activated by 7 intracellular ligands but also by extracellular stimuli, such as angiotensin II, TNF-α and other cytokines (278) - Widely expressed in glomeruli and tubules (278) - Important role in nephrogenesis, electrolyte handling, and tissue repair (especially following acute kidney injury): may be a surrogate marker for regenerative tubular function reserve (278, 279) - Pathway also involved in the initiation and progression of various chronic kidney diseases through inflammation, fibrosis and cellular proliferation (278, 279)
ICAM-1	- Adhesion molecule playing a role in inflammation and regulation of vascular permeability, promotes leukocytes-endothelium interactions (280, 281) - In IgAVN, serum levels increased in the active phase compared to convalescent phase of the disease, but no statistical difference from healthy and disease-control groups (214, 215, 282, 283) - Mesangial expression in IgAVN and IgA nephropathy reported (215) - Anti-endothelial cell antibodies may upregulate ICAM-1, further contributing to vascular damage (see **1.1.3.4**)
CXCL16	- Chemokine mainly involved in leukocytes migration (284) - CXCL16 induced by angiotensin II in renal tubular epithelial cells upregulate hypertensive renal injury and fibrosis in an NFκB dependent manner (285, 286) - Plasma levels correlated with clinical and histological features, extent of infiltrating inflammatory cells and renal outcome in 230 children with IgA nephropathy (287)
CXCL1	- Chemokine, powerful neutrophil chemoattractant (284) - In mesangial cells, upregulation induced by IgA1 complexes from IgA nephropathy patients *in vitro*, with resultant podocyte injury (288) - High urinary levels associated with severity of clinical and pathological features and poor renal prognosis in adults with IgA nephropathy (289)
IGFBP-3	- Binds insulin-like growth factor-1 (IGF-1), a hormone essential for normal growth and metabolism regulation (290) - Over-expression associated with the development of renal diseases, such as mesangial proliferation and podocytosis in animal models of IgA nephropathy (291) - However, unclear whether IGFBP-3 is involved in the pathophysiology of CKD
ST2	- Member of IL-1 receptor family, induces inflammation by inhibiting IL-33/ST2L signalling (127) - Important prognostic marker in patients with heart failure, including in those with CKD (127)

ST2 (*continued*)	-	Higher serum levels and correlated with the severity of IgA nephropathy (292), however a study reported no difference in 33 patients with IgAV (of which 36.4% had IgAVN) (293)
CD40L	-	Primarily expressed by activated T cells, activated B-cells, and platelets. Also exists in soluble form (294)
	-	CD40/CD40L signalling plays important role in the progression of a range of renal diseases (294)
	-	May play a role in the differentiation and functional maturation of B10 cells in children with IgAVN (295)
	-	Can activate endothelial cells leading to reactive oxygen species, chemokine and cytokine production, in addition to expression of adhesions molecules E-selectin, ICAM-1, and VCAM-1 (296), further promoting vascular inflammation (see **1.1.3.4**)
Serpin E1	-	Serine protease inhibiting plasminogen activator, thus inhibiting fibrinolysis (297)
	-	Intrarenal production in states of acute and chronic disease, and by angiotensin II (297)
	-	Plasma levels correlate with disease with renal disease severity and long-term outcome (297)
	-	Powerful fibrosis-promoting effect on the kidneys (297)
	-	RAAS inhibition suppressed the angiotensin II-induced serpin E1 in mesangial cells *in vitro*, hence it may be another novel therapeutic target (298, 299)
LIF	-	Member of the IL-6 family, synthesised by a variety of cells including renal epithelial cells (300)
	-	Involved in kidney development, inflammation and renal regeneration following tubular injury (300)
	-	Enhanced production of gd-IgA1 *in vitro* (301)
IL-5	-	Th2-type cytokine
	-	Important role in supporting the growth and differentiation of B cells, especially by promoting B cell differentiation into IgA-secreting cells (302)
	-	In IgA nephropathy, plasma IL-5 stimulated the production of abnormally glycolysated IgA *in vitro* and in animal models (302)

Table 4.5*: Other urinary proteins significantly increased in IgAVN vs. IgAVwoN that had not been reported in the literature before for IgAVN. EGF-R: epithelial growth factor receptor; ICAM-1: intracellular adhesion molecule 1; CXCL16: CXC motif cytokine ligand 16; CXCL1: CXC motif cytokine ligand 1; IGFBP-3; insulin-like growth factor-binding protein 3; ST2: suppression of tumorigenicity 2; CD40L: cluster of differentiation 40 ligand; RAAS: renin-angiotensin aldosterone system; LIF: leukaemia inhibitory factor: IL: interleukin.*

4.4.8. Already known markers of IgAVN

Our findings are consistent with those reported in a recent systematic literature review that identified urinary biomarkers in children with IgAVN (128). The current summary of the literature supported the significance of UAGT, β2-microglobulin, KIM-1 and MCP-1 (128). In addition, individual

studies have reported elevated urinary levels of MIF (303), MMP-9 (304), IL-6, IL-8 and IL-10 (195) in paediatric IgAVN. Our study did not find elevated levels of β2-microglobulin, MMP-9 and IL-6/8/10 in IgAVN, however it is important to note that this was a small preliminary study using a semi-quantitative technique. Dyga and colleagues did not find any significant association between the concentrations of urinary NGAL and L-FABP (305), which is in keeping with our findings. Our findings of elevated urinary proteins which could result from tubulointerstitial inflammation/injury (KIM-1, ApoA1, EGF-R, AGT, LIF) further supports that IgAVN includes some degree of tubular inflammation in addition to the glomerulonephritis, as previously proposed by Williams *et al* (128).

4.4.9. Limitations

This study has several limitations, first of all due to its cross-sectional nature, with samples obtained at different stages of the disease. The assays were semi-quantitative and despite good agreement in general between the inflammatory markers present in both kits, there were minor fold-change and *p*-value differences of the duplicated proteins between kits K and C.

Finally, it remains unclear whether these markers are specific to IgAVN or result from pathophysiological features shared between different renal diseases, such as excessive inflammation, or a by-product of proteinuria. While this study was limited in terms of sample size, it provides exploratory data to direct future studies, and it demonstrates that urinary protein profiles do vary between IgAV and IgAVN patients. Future work will look to utilise more robust quantitative methods, such as ELISAs and mass spectrometry techniques, to validate the findings of the most highly expressed proteins using a larger patient cohort.

4.5. Conclusions

The current study utilised a small number of patients in a cross-sectional design to explore a large number of urinary proteins in patients with established IgAVN. It was able to uncover some interesting variations in the urinary protein profiles that may help elucidate further mechanisms of pathophysiology and represent potential drug targets for intervention. Furthermore, longitudinal evaluation would allow for the development of nephritis to be better characterised, which would significantly improve our mechanistic understanding of the development of IgAVN.

5. Discussion

As IgAV is a self-limiting disease in the vast majority of patients, it is often perceived as a disease with low disease activity and this can add frustration and potential delays in patients with atypical features reaching specialist care. This is further exacerbated by the lack of standardised definitions and consensus on the management of more complex cases, many of whom may be at increased risk of nephritis. It is well recognised that progression to CKD 5 is reported in 1-2% of children, however in the very long term, severe renal impairment can occur in 35% of adults who had IgAVN in childhood (19, 102). In view of these circumstances, the overall aim of this thesis was to improve the understanding of disease activity in children with IgAV.

The first section of this work allowed potential standard definitions for recurrent and persisting IgAV following an evaluation of a clinical cohort and an extensive review of the literature, which will provide a framework for further discussion as part of a national working group to help develop the first national best-practice clinical guidelines for the diagnosis and management of IgAV in children. Next, the second chapter managed to determine that IgA and C5a could be measured in the urine and may be indicative of nephritis in children with IgAV. Finally, the last section of this thesis involved a broad approach to analyse multiple protein markers to provide insight into the pathophysiology of IgAVN. This exploratory data is an important step that could lead to early stratification of the high-risk patients, following validation in large longitudinal prospective studies. This is essential to be able to utilise what patients and their families often describe as a "window of opportunity" for early clinical intervention or closer monitoring. This work demonstrates that urine could be an informative non-invasive biofluid for future large longitudinal prospective studies to investigate the correlation of the identified markers to clinical characteristics, explore potential drug targets and improve patient outcomes.

Overall this thesis has provided some insight into achieving standardised ways to evaluate IgAV and a better understanding of the pathophysiology leading to nephritis.

5.1. Limitations

This thesis has limitations that have been discussed in each chapter. Overall, the main limitation includes the fact that this work retrospectively collected data from a tertiary centre which is more likely to treat patients with a more severe disease course. This is particularly relevant for the children in the case series, as many were referred by other healthcare providers. It may be that the children with milder recurrences were managed in primary/secondary care, hence our population was skewed.

Another limitation of this work is the sample size in both chapters 2 and 4, as larger cohorts are needed to validate the findings. Similarly, the IgAV study was set up as a pragmatic study to initially collect cross-sectional samples, hence the heterogenicity in the patient populations. In addition, chapter 4 was based on a semi-quantitative technique, and although it provides interesting exploratory data, quantitative validation of these findings is needed. Finally, the addition of an autoimmune control group may have improved this work.

5.2. Recent initiatives and future work

In addition to enhancing the understanding of paediatric IgAV disease activity, this work has provided pilot data and opens up new areas for future work. Chapter 2 specifically highlighted the lack of standardisation in this disease, which may be one reason contributing to the lack of high-quality evidence. Recently, the SHARE guidelines aimed to provide internationally agreed consensus recommendations for the diagnosis and treatment of children with IgAV. However, no recommendations were issued for patients with a more complex disease course. Already, national and international initiatives are being undertaken: nationally, the development of two NICE-endorsed best practice clinical guidelines for the diagnosis and management of IgAV in children should provide a solid foundation for both clinical practice and future potential clinical trials. Internationally, the WORLD-IgAVN project ('Working together to agree, Lead and Define core features of a clinical trial in Immunoglobulin A vasculitis nephritis') will establish a working group to provide a structure for future studies and enhance international collaboration.

Chapters 3 and 4 uncovered some new interesting urinary proteins that had not been reported before in children with IgAVN. Specifically, the role of B-cell activation, the complement system and the RAAS system should be further explored, due to their potential use as a therapeutic target and the many ongoing clinical trials in similar diseases, especially in IgA nephropathy. They could also be useful markers of disease activity. Future work is already underway to validate these findings using quantitative methods. Further, the structure of the urinary IgA should be explored, especially to characterise any relationship between IgA glycan-specificity and the renal phenotype. Large multicentre longitudinal studies, using remote sampling to recruit patients from the time of diagnosis, are needed to investigate the timing of onset and prognostic value of large biomarker panels; this could be achieved using multiplexing Luminex assays or mass spectrometry techniques.

6. Conclusions

There are unmet needs in many aspects of managing paediatric IgA vasculitis. This work evaluated children with a recurrent and/or a prolonged disease course to propose standardised definitions and contributed to the discovery of new urinary markers of kidney inflammation providing novel insight into the pathophysiology of IgAV nephritis. Further translational research studies, randomised clinical trials and standardised clinical guidelines are urgently needed to improve outcomes in this disease.

7. References

1. Gardner-Medwin JM, Dolezalova P, Cummins C, Southwood TR. Incidence of Henoch-Schönlein purpura, Kawasaki disease, and rare vasculitides in children of different ethnic origins. Lancet. 2002 Oct 19;360(9341):1197-202. eng. Epub 2002/10/29. doi:10.1016/s0140-6736(02)11279-7. Cited in: Pubmed; PMID 12401245.
2. Jennette JC, Falk RJ, Bacon PA, Basu N, Cid MC, Ferrario F, Flores-Suarez LF, Gross WL, Guillevin L, Hagen EC, Hoffman GS, Jayne DR, Kallenberg CG, Lamprecht P, Langford CA, Luqmani RA, Mahr AD, Matteson EL, Merkel PA, Ozen S, Pusey CD, Rasmussen N, Rees AJ, Scott DG, Specks U, Stone JH, Takahashi K, Watts RA. 2012 revised International Chapel Hill Consensus Conference Nomenclature of Vasculitides. Arthritis Rheum. 2013 Jan;65(1):1-11. eng. Epub 2012/10/10. doi:10.1002/art.37715. Cited in: Pubmed; PMID 23045170.
3. Rai A, Nast C, Adler S. Henoch-Schönlein Purpura Nephritis. Journal of the American Society of Nephrology. 1999;10(12):2637. doi:10.1681/ASN.V10122637.
4. Leung AKC, Barankin B, Leong KF. Henoch-Schönlein Purpura in Children: An Updated Review. Curr Pediatr Rev. 2020;16(4):265-276. eng. Epub 2020/05/10. doi:10.2174/1573396316666200508104708. Cited in: Pubmed; PMID 32384035.
5. Piram M, Mahr A. Epidemiology of immunoglobulin A vasculitis (Henoch-Schönlein): current state of knowledge. Curr Opin Rheumatol. 2013 Mar;25(2):171-8. eng. Epub 2013/01/16. doi:10.1097/BOR.0b013e32835d8e2a. Cited in: Pubmed; PMID 23318735.
6. Tracy A, Subramanian A, Adderley NJ, Cockwell P, Ferro C, Ball S, Harper L, Nirantharakumar K. Cardiovascular, thromboembolic and renal outcomes in IgA vasculitis (Henoch-Schönlein purpura): a retrospective cohort study using routinely collected primary care data. Annals of the Rheumatic Diseases. 2019;78(2):261. doi:10.1136/annrheumdis-2018-214142.
7. Chan H, Tang YL, Lv XH, Zhang GF, Wang M, Yang HP, Li Q. Risk Factors Associated with Renal Involvement in Childhood Henoch-Schönlein Purpura: A Meta-Analysis. PLoS One. 2016;11(11):e0167346. eng. Epub 2016/12/03. doi:10.1371/journal.pone.0167346. Cited in: Pubmed; PMID 27902749.
8. Shin JI, Park JM, Shin YH, Hwang DH, Kim JH, Lee JS. Predictive factors for nephritis, relapse, and significant proteinuria in childhood Henoch-Schönlein purpura. Scand J Rheumatol. 2006 Jan-Feb;35(1):56-60. eng. Epub 2006/02/10. doi:10.1080/03009740510026841. Cited in: Pubmed; PMID 16467044.
9. Jauhola O, Ronkainen J, Koskimies O, Ala-Houhala M, Arikoski P, Hölttä T, Jahnukainen T, Rajantie J, Örmälä T, Nuutinen M. Clinical course of extrarenal symptoms in Henoch–Schönlein purpura: a 6-month prospective study. Archives of Disease in Childhood. 2010;95(11):871. doi:10.1136/adc.2009.167874.
10. Audemard-Verger A, Pillebout E, Baldolli A, Gouellec NL, Augusto JF, Jourde-Chiche N, Raffray L, Thervet E, Deroux A, Goutte J, Hummel A, Lioger B, Sanges S, Cacoub P, Amoura Z, Moulis G, Maurier F, Lavigne C, Urbanski G, Chanal J, Faguer S, Deriaz S, Feirreira-Maldent N, Diot E, Maillot F, Guillevin L, Terrier B. Impact of aging on phenotype and prognosis in IgA vasculitis. Rheumatology (Oxford). 2021 Sep 1;60(9):4245-4251. eng. Epub 2021/01/08. doi:10.1093/rheumatology/keaa921. Cited in: Pubmed; PMID 33410479.
11. Shi D, Chan H, Yang X, Zhang G, Yang H, Wang M, Li Q. Risk factors associated with IgA vasculitis with nephritis (Henoch-Schönlein purpura nephritis) progressing to unfavorable outcomes: A meta-analysis. PLoS One. 2019;14(10):e0223218. eng. Epub 2019/10/02. doi:10.1371/journal.pone.0223218. Cited in: Pubmed; PMID 31574112.
12. Liao C-H, Tsai M, Yang Y-H, Chiang B-L, Wang L-C. Onset age is a risk factor for refractory pediatric IgA vasculitis: a retrospective cohort study. Pediatric Rheumatology. 2020 2020/11/10;18(1):86. doi:10.1186/s12969-020-00480-3.
13. Shim JO, Han K, Park S, Kim GH, Ko JS, Chung JY. Ten-year Nationwide Population-based Survey on the Characteristics of Children with Henoch-Schönlein Purpura in Korea. J Korean Med Sci. 2018 Jun 18;33(25):e174. eng. Epub 2018/06/20. doi:10.3346/jkms.2018.33.e174. Cited in: Pubmed; PMID 29915525.
14. Lei W-T, Tsai P-L, Chu S-H, Kao Y-H, Lin C-Y, Fang L-C, Shyur S-D, Lin Y-W, Wu S-I. Incidence and risk factors for recurrent Henoch-Schönlein purpura in children from a 16-year nationwide database. Pediatric Rheumatology. 2018 2018/04/16;16(1):25. doi:10.1186/s12969-018-0247-8.
15. Yang YH, Hung CF, Hsu CR, Wang LC, Chuang YH, Lin YT, Chiang BL. A nationwide survey on epidemiological characteristics of childhood Henoch-Schönlein purpura in Taiwan. Rheumatology (Oxford). 2005 May;44(5):618-22. eng. Epub 2005/01/27. doi:10.1093/rheumatology/keh544. Cited in: Pubmed; PMID 15671050.
16. Piram M, Maldini C, Biscardi S, De Suremain N, Orzechowski C, Georget E, Regnard D, Koné-Paut I, Mahr A. Incidence of IgA vasculitis in children estimated by four-source capture-recapture analysis: a population-based study. Rheumatology (Oxford). 2017 Aug 1;56(8):1358-1366. eng. Epub 2017/04/27. doi:10.1093/rheumatology/kex158. Cited in: Pubmed; PMID 28444335.
17. Mossberg M, Segelmark M, Kahn R, Englund M, Mohammad AJ. Epidemiology of primary systemic vasculitis in children: a population-based study from southern Sweden. Scand J Rheumatol. 2018 Jul;47(4):295-302. eng. Epub 2018/02/08. doi:10.1080/03009742.2017.1412497. Cited in: Pubmed; PMID 29409373.
18. Penny K, Fleming M, Kazmierczak D, Thomas A. An epidemiological study of Henoch-Schönlein purpura. Paediatr Nurs. 2010 Dec;22(10):30-5. eng. Epub 2011/02/08. doi:10.7748/paed2010.12.22.10.30.c8135. Cited in: Pubmed; PMID 21294500.
19. Oni L, Sampath S. Childhood IgA Vasculitis (Henoch Schonlein Purpura)-Advances and Knowledge Gaps. Front Pediatr. 2019;7:257. eng. Epub 2019/07/19. doi:10.3389/fped.2019.00257. Cited in: Pubmed; PMID 31316952.
20. Lévy M. [Familial cases of Berger's disease or of Berger's disease and rheumatoid purpura. Cooperative study of the Société Française de Néphrologie]. Nephrologie. 1989;10 4:175-82.
21. Kiryluk K, Moldoveanu Z, Sanders JT, Eison TM, Suzuki H, Julian BA, Novak J, Gharavi AG, Wyatt RJ. Aberrant glycosylation of IgA1 is inherited in both pediatric IgA nephropathy and Henoch–Schönlein purpura nephritis. Kidney International. 2011 2011/07/01/;80(1):79-87. doi:https://doi.org/10.1038/ki.2011.16.
22. Boyd JK, Barratt J. Inherited IgA glycosylation pattern in IgA nephropathy and HSP nephritis: where do we go next? Kidney International. 2011 2011/07/01/;80(1):8-10. doi:https://doi.org/10.1038/ki.2011.83.
23. Hastings MC, Rizk DV, Kiryluk K, Nelson R, Zahr RS, Novak J, Wyatt RJ. IgA vasculitis with nephritis: update of pathogenesis with clinical implications. Pediatr Nephrol. 2021 Apr 5. eng. Epub 2021/04/06. doi:10.1007/s00467-021-04950-y. Cited in: Pubmed; PMID 33818625.
24. Zhang Y, Gu W, Mao J. Sibling Cases of Henoch–Schönlein Purpura in Two Families and Review of Literature [https://doi.org/10.1111/j.1525-1470.2008.00693.x]. Pediatric Dermatology. 2008 2008/05/01;25(3):393-395. doi:https://doi.org/10.1111/j.1525-1470.2008.00693.x.
25. López-Mejías R, Carmona FD, Castañeda S, Genre F, Remuzgo-Martínez S, Sevilla-Perez B, Ortego-Centeno N, Llorca J, Ubilla B, Mijares V, Pina T, Miranda-Filloy JA, Navas Parejo A, de Argila D, Aragües M, Rubio E, Luque ML, Blanco-Madrigal JM, Galíndez-Aguirregoikoa E, Jayne D,

Blanco R, Martín J, González-Gay MA. A genome-wide association study suggests the HLA Class II region as the major susceptibility locus for IgA vasculitis. Sci Rep. 2017 Jul 11;7(1):5088. eng. Epub 2017/07/13. doi:10.1038/s41598-017-03915-2. Cited in: Pubmed; PMID 28698626.
26. Koskela M, Nihtilä J, Ylinen E, Kolho K-L, Nuutinen M, Ritari J, Jahnukainen T. HLA-DQ and HLA-DRB1 alleles associated with Henoch-Schönlein purpura nephritis in Finnish pediatric population: a genome-wide association study. Pediatric Nephrology. 2021 2021/08/01;36(8):2311-2318. doi:10.1007/s00467-021-04955-7.
27. López-Mejías R, Castañeda S, Genre F, Remuzgo-Martínez S, Carmona FD, Llorca J, Blanco R, Martín J, González-Gay MA. Genetics of immunoglobulin-A vasculitis (Henoch-Schönlein purpura): An updated review. Autoimmunity Reviews. 2018 2018/03/01/;17(3):301-315. doi:https://doi.org/10.1016/j.autrev.2017.11.024.
28. Abbara S, Grateau G, Ducharme-Bénard S, Saadoun D, Georgin-Lavialle S. Association of Vasculitis and Familial Mediterranean Fever. Front Immunol. 2019;10:763-763. eng. doi:10.3389/fimmu.2019.00763. Cited in: Pubmed; PMID 31031761.
29. Jelusic M, Sestan M. IgA vasculitis or Henoch-Schönlein purpura: genetics and beyond. Pediatric Nephrology. 2021 2021/08/01;36(8):2149-2153. doi:10.1007/s00467-021-04987-z.
30. Research NIfHaC. NIHR BioResource Home Page. 2022 18/06/2022. Available from: https://bioresource.nihr.ac.uk/.
31. Hwang HH, Lim IS, Choi B-S, Yi DY. Analysis of seasonal tendencies in pediatric Henoch–Schönlein purpura and comparison with outbreak of infectious diseases. Medicine (Baltimore). 2018;97(36).
32. Weiss PF, Klink AJ, Luan X, Feudtner C. Temporal association of Streptococcus, Staphylococcus, and parainfluenza pediatric hospitalizations and hospitalized cases of Henoch-Schönlein purpura. J Rheumatol. 2010 Dec;37(12):2587-94. eng. Epub 2010/09/17. doi:10.3899/jrheum.100364. Cited in: Pubmed; PMID 20843903.
33. Rigante D, Castellazzi L, Bosco A, Esposito S. Is there a crossroad between infections, genetics, and Henoch–Schönlein purpura? Autoimmunity Reviews. 2013 2013/08/01/;12(10):1016-1021. doi:https://doi.org/10.1016/j.autrev.2013.04.003.
34. Farooq H, Aemaz Ur Rehman M, Asmar A, Asif S, Mushtaq A, Qureshi MA. The pathogenesis of COVID-19-induced IgA nephropathy and IgA vasculitis: A systematic review. Journal of Taibah University Medical Sciences. 2022 2022/02/01/;17(1):1-13. doi:https://doi.org/10.1016/j.jtumed.2021.08.012.
35. Yan M, Wang Z, Niu N, Zhao J, Peng J. Relationship between chronic tonsillitis and Henoch-Schonlein purpura. Int J Clin Exp Med. 2015;8(8):14060-14064. eng. Cited in: Pubmed; PMID 26550368.
36. Holder SMt, Joy MS, Falk RJ. Cutaneous and Systemic Manifestations of Drug-Induced Vasculitis. Annals of Pharmacotherapy. 2002 2002/01/01;36(1):130-147. doi:10.1345/aph.1A124.
37. Oni L. IgA vasculitis (Henoch-Schonlein purpura). BMJ Best Practice; 2021 Accessed 07/03/2022. [11 June 2021]. Available from: https://bestpractice.bmj.com/topics/en-gb/110.
38. Rasmussen C, Tisseyre M, Garon-Czmil J, Atzenhoffer M, Guillevin L, Salem J-E, Treluyer J-M, Terrier B, Chouchana L. Drug-induced IgA vasculitis in children and adults: Revisiting drug causality using a dual pharmacovigilance-based approach. Autoimmunity Reviews. 2021 2021/01/01/;20(1):102707. doi:https://doi.org/10.1016/j.autrev.2020.102707.
39. Da Dalt L, Zerbinati C, Strafella MS, Renna S, Riceputi L, Di Pietro P, Barabino P, Scanferla S, Raucci U, Mores N, Compagnone A, Da Cas R, Menniti-Ippolito F, Menniti-Ippolito F, Da Cas R, Traversa G, Santuccio C, Felicetti P, Tartaglia L, Trotta F, Di Pietro P, Barabino P, Renna S, Riceputi L, Tovo P-A, Gabiano C, Urbino A, Baroero L, Le Serre D, Virano S, Da Dalt L, Stefani C, Zerbinati C, Perilongo G, Daverio M, Maretti M, Galeazzo B, Rubin G, Scanferla S, Chiappini E, Sollai S, De Martino M, Becciani S, Giacalone M, Montano S, Remaschi G, Stival A, Abate P, Leonardi I, Pirozzi N, Raucci U, Reale A, Rossi R, Mores N, Bersani G, Compagnone A, Chiaretti A, Riccardi R, Romagnoli C, Tipo V, Dinardo M, Auricchio F, Polimeno T, Bonagura MC, Maccariello A, Fucà F, Di Rosa E, Altavilla D, Mecchio A, Arrigo T, the Italian Multicenter Study Group for D, Vaccine Safety in C. Henoch-Schönlein purpura and drug and vaccine use in childhood: a case-control study. Italian Journal of Pediatrics. 2016 2016/06/18;42(1):60. doi:10.1186/s13052-016-0267-2.
40. Piram M, Gonzalez Chiappe S, Madhi F, Ulinski T, Mahr A. Vaccination and Risk of Childhood IgA Vasculitis. Pediatrics. 2018;142(5):e20180841. doi:10.1542/peds.2018-0841.
41. Sapina M, Frkovic M, Sestan M, Srsen S, Ovuka A, Batnozic Varga M, Kramaric K, Brdaric D, Milas K, Gagro A, Jelusic M. Geospatial clustering of childhood IgA vasculitis and IgA vasculitis-associated nephritis. Annals of the Rheumatic Diseases. 2021;80(5):610. doi:10.1136/annrheumdis-2020-218649.
42. Puntarić D, Bosnir J, Smit Z, Skes I, Baklaić Z. Ochratoxin A in corn and wheat: geographical association with endemic nephropathy. Croat Med J. 2001 Apr;42(2):175-80. eng. Epub 2001/03/22. Cited in: Pubmed; PMID 11259741.
43. Breedveld A, van Egmond M. IgA and FcαRI: Pathological Roles and Therapeutic Opportunities [Review]. Front Immunol. 2019 2019-March-22;10. English. doi:10.3389/fimmu.2019.00553.
44. Song Y, Huang X, Yu G, Qiao J, Cheng J, Wu J, Chen J. Pathogenesis of IgA Vasculitis: An Up-To-Date Review [Mini Review]. Front Immunol. 2021 2021-November-09;12. English. doi:10.3389/fimmu.2021.771619.
45. Heineke MH, Ballering AV, Jamin A, Ben Mkaddem S, Monteiro RC, Van Egmond M. New insights in the pathogenesis of immunoglobulin A vasculitis (Henoch-Schönlein purpura). Autoimmunity Reviews. 2017 2017/12/01/;16(12):1246-1253. doi:https://doi.org/10.1016/j.autrev.2017.10.009.
46. Suzuki H, Yasutake J, Makita Y, Tanbo Y, Yamasaki K, Sofue T, Kano T, Suzuki Y. IgA nephropathy and IgA vasculitis with nephritis have a shared feature involving galactose-deficient IgA1-oriented pathogenesis. Kidney Int. 2018 Mar;93(3):700-705. eng. Epub 2018/01/14. doi:10.1016/j.kint.2017.10.019. Cited in: Pubmed; PMID 29329643.
47. Sugino H, Sawada Y, Nakamura M. IgA Vasculitis: Etiology, Treatment, Biomarkers and Epigenetic Changes. International Journal of Molecular Sciences. 2021;22(14). doi:10.3390/ijms22147538.
48. Neufeld M, Molyneux K, Pappelbaum KI, Mayer-Hain S, von Hodenberg C, Ehrchen J, Barratt J, Suzuki Y, Sunderkötter C. Galactose-deficient IgA1 in skin and serum from patients with skin-limited and systemic IgA vasculitis. J Am Acad Dermatol. 2019 Nov;81(5):1078-1085. eng. Epub 2019/03/25. doi:10.1016/j.jaad.2019.03.029. Cited in: Pubmed; PMID 30902725.
49. Legendre P, Régent A, Thiebault M, Mouthon L. Anti-endothelial cell antibodies in vasculitis: A systematic review. Autoimmunity Reviews. 2017 2017/02/01/;16(2):146-153. doi:https://doi.org/10.1016/j.autrev.2016.12.012.
50. Rizk DV, Maillard N, Julian BA, Knoppova B, Green TJ, Novak J, Wyatt RJ. The Emerging Role of Complement Proteins as a Target for Therapy of IgA Nephropathy [Review]. Front Immunol. 2019 2019-March-19;10. English. doi:10.3389/fimmu.2019.00504.
51. Merle NS, Church SE, Fremeaux-Bacchi V, Roumenina LT. Complement System Part I – Molecular Mechanisms of Activation and Regulation [Review]. Front Immunol. 2015 2015-June-02;6. English. doi:10.3389/fimmu.2015.00262.
52. Noris M, Remuzzi G. Overview of Complement Activation and Regulation. Seminars in Nephrology. 2013 2013/11/01/;33(6):479-492. doi:https://doi.org/10.1016/j.semnephrol.2013.08.001.

53. Barratt J, Weitz I. Complement Factor D as a Strategic Target for Regulating the Alternative Complement Pathway. Front Immunol. 2021;12:712572-712572. eng. doi:10.3389/fimmu.2021.712572. Cited in: Pubmed; PMID 34566967.
54. Damman J, Mooyaart AL, Bosch TPPvd, Seelen MAJ, Doorn MBAv. Lectin and alternative complement pathway activation in cutaneous manifestations of IgA-vasculitis: A new target for therapy? Molecular Immunology. 2022 2022/03/01/;143:114-121. doi:https://doi.org/10.1016/j.molimm.2022.01.011.
55. Endo M, Ohi H, Ohsawa I, Fujita T, Matsushita M, Fujita T. Complement activation through the lectin pathway in patients with Henoch-Schönlein purpura nephritis. American Journal of Kidney Diseases. 2000 2000/03/01/;35(3):401-407. doi:https://doi.org/10.1016/S0272-6386(00)70192-2.
56. Hisano S, Matsushita M, Fujita T, Iwasaki H. Activation of the lectin complement pathway in Henoch-Schönlein purpura nephritis. American Journal of Kidney Diseases. 2005 2005/02/01/;45(2):295-302. doi:https://doi.org/10.1053/j.ajkd.2004.10.020.
57. Yang Y-H, Tsai IJ, Chang C-J, Chuang Y-H, Hsu H-Y, Chiang B-L. The Interaction between Circulating Complement Proteins and Cutaneous Microvascular Endothelial Cells in the Development of Childhood Henoch-Schönlein Purpura. PLOS ONE. 2015;10(3):e0120411. doi:10.1371/journal.pone.0120411.
58. Jia M, Zhu L, Zhai YL, Chen P, Xu BY, Guo WY, Shi SF, Liu LJ, Lv JC, Zhang H. Variation in complement factor H affects complement activation in immunoglobulin A vasculitis with nephritis. Nephrology (Carlton). 2020 Jan;25(1):40-47. eng. Epub 2019/03/07. doi:10.1111/nep.13580. Cited in: Pubmed; PMID 30838755.
59. Chua JS, Zandbergen M, Wolterbeek R, Baelde HJ, van Es LA, de Fijter JW, Bruijn JA, Bajema IM. Complement-mediated microangiopathy in IgA nephropathy and IgA vasculitis with nephritis. Modern Pathology. 2019 2019/08/01;32(8):1147-1157. doi:10.1038/s41379-019-0259-z.
60. Girardi G, Lingo JJ, Fleming SD, Regal JF. Essential Role of Complement in Pregnancy: From Implantation to Parturition and Beyond [Review]. Front Immunol. 2020 2020-July-31;11. English. doi:10.3389/fimmu.2020.01681.
61. Su Z, Lv X, Liu Y, Zhang J, Guan J, Gai Z. Corrigendum to "Circulating midkine in children with Henoch-Schönlein purpura: Clinical implications" [Int. Immunopharmacol. (2016) 246–250]. International Immunopharmacology. 2016 2016/12/01/;41:145. doi:https://doi.org/10.1016/j.intimp.2016.08.039.
62. Su Z, Lv X, Liu Y, Zhang J, Guan J, Gai Z. Circulating midkine in children with Henoch-Schönlein purpura: Clinical implications. International Immunopharmacology. 2016 2016/10/01/;39:246-250. doi:https://doi.org/10.1016/j.intimp.2016.07.032.
63. Jen HY, Chuang YH, Lin SC, Chiang BL, Yang YH. Increased serum interleukin-17 and peripheral Th17 cells in children with acute Henoch-Schönlein purpura. Pediatr Allergy Immunol. 2011 Dec;22(8):862-8. eng. Epub 2011/09/21. doi:10.1111/j.1399-3038.2011.01198.x. Cited in: Pubmed; PMID 21929599.
64. Audemard-Verger A, Pillebout E, Jamin A, Berthelot L, Aufray C, Martin B, Sannier A, Daugas E, Déchanet-Merville J, Richard Y, Monteiro R, Lucas B. Recruitment of CXCR3(+) T cells into injured tissues in adult IgA vasculitis patients correlates with disease activity. J Autoimmun. 2019 May;99:73-80. eng. Epub 2019/02/13. doi:10.1016/j.jaut.2019.01.012. Cited in: Pubmed; PMID 30745186.
65. Imai T, Nishiyama K, Ueki K, Tanaka T, Kaku Y, Hara T, Ohga S. Involvement of activated cytotoxic T lymphocytes and natural killer cells in Henoch–Schönlein purpura nephritis [https://doi.org/10.1002/cti2.1212]. Clinical & Translational Immunology. 2020 2020/01/01;9(11):e1212. doi:https://doi.org/10.1002/cti2.1212.
66. Du L, Wang P, Liu C, Li S, Yue S, Yang Y. Multisystemic manifestations of IgA vasculitis. Clinical Rheumatology. 2021 2021/01/01;40(1):43-52. doi:10.1007/s10067-020-05166-5.
67. CalviÑO MC, Llorca J, GarcÍA-PorrÚA C, FernÁNdez-Iglesias JL, Rodriguez-Ledo P, GonzÁLez-Gay MA. Henoch-Schönlein Purpura in Children from Northwestern Spain: A 20-Year Epidemiologic and Clinical Study. Medicine (Baltimore). 2001;80(5).
68. Saulsbury FT. Clinical update: Henoch-Schönlein purpura. Lancet. 2007 Mar 24;369(9566):976-8. eng. Epub 2007/03/27. doi:10.1016/s0140-6736(07)60474-7. Cited in: Pubmed; PMID 17382810.
69. Rubino C, Monacelli C, Marrani E, Paci M, Indolfi G, Simonini G, Trapani S. Gastrointestinal involvement in IgA vasculitis: a single-center 11-year study on a cohort of 118 children. Clinical Rheumatology. 2021 2021/12/01;40(12):5041-5046. doi:10.1007/s10067-021-05863-9.
70. Nong BR, Huang YF, Chuang CM, Liu CC, Hsieh KS. Fifteen-year experience of children with Henoch-Schönlein purpura in southern Taiwan, 1991-2005. J Microbiol Immunol Infect. 2007 Aug;40(4):371-6. eng. Epub 2007/08/23. Cited in: Pubmed; PMID 17712473.
71. Chen SY, Kong MS. Gastrointestinal manifestations and complications of Henoch-Schönlein purpura. Chang Gung Med J. 2004 Mar;27(3):175-81. eng. Epub 2004/05/20. Cited in: Pubmed; PMID 15148994.
72. Ebert EC. Gastrointestinal manifestations of Henoch-Schonlein Purpura. Dig Dis Sci. 2008 Aug;53(8):2011-9. eng. Epub 2008/03/21. doi:10.1007/s10620-007-0147-0. Cited in: Pubmed; PMID 18351468.
73. Nikibakhsh AA, Mahmoodzadeh H, Karamyyar M, Hejazi S, Noroozi M, Macooie AA. Treatment of severe henoch-schonlein purpura nephritis with mycophenolate mofetil. Saudi J Kidney Dis Transpl. 2014 Jul;25(4):858-63. eng. Epub 2014/06/28. doi:10.4103/1319-2442.135182. Cited in: Pubmed; PMID 24969202.
74. Narchi H. Risk of long term renal impairment and duration of follow up recommended for Henoch-Schonlein purpura with normal or minimal urinary findings: a systematic review. Archives of disease in childhood. 2005;90(9):916-920. eng. Epub 2005/05/04. doi:10.1136/adc.2005.074641. Cited in: Pubmed; PMID 15871983.
75. Modi S, Mohan M, Jennings A. Acute Scrotal Swelling in Henoch-Schonlein Purpura: Case Report and Review of the Literature. Urology Case Reports. 2016 2016/05/01/;6:9-11. doi:https://doi.org/10.1016/j.eucr.2016.01.004.
76. Hara Y, Tajiri T, Matsuura K, Hasegawa A. Acute scrotum caused by Henoch-Schönlein purpura. International Journal of Urology. 2004;11(7):578-580. doi:10.1111/j.1442-2042.2004.00835.x.
77. Rajagopala S, Shobha V, Devaraj U, D'Souza G, Garg I. Pulmonary hemorrhage in Henoch-Schönlein purpura: case report and systematic review of the english literature. Semin Arthritis Rheum. 2013 Feb;42(4):391-400. eng. Epub 2012/09/19. doi:10.1016/j.semarthrit.2012.07.004. Cited in: Pubmed; PMID 22985708.
78. Ozen S, Marks SD, Brogan P, Groot N, de Graeff N, Avcin T, Bader-Meunier B, Dolezalova P, Feldman BM, Kone-Paut I, Lahdenne P, McCann L, Pilkington C, Ravelli A, van Royen A, Uziel Y, Vastert B, Wulffraat N, Kamphuis S, Beresford MW. European consensus-based recommendations for diagnosis and treatment of immunoglobulin A vasculitis-the SHARE initiative. Rheumatology (Oxford). 2019 Sep 1;58(9):1607-1616. eng. Epub 2019/03/18. doi:10.1093/rheumatology/kez041. Cited in: Pubmed; PMID 30879080.
79. Ozen S, Pistorio A, Iusan SM, Bakkaloglu A, Herlin T, Brik R, Buoncompagni A, Lazar C, Bilge I, Uziel Y, Rigante D, Cantarini L, Hilario MO, Silva CA, Alegria M, Norambuena X, Belot A, Berkun Y, Estrella AI, Olivieri AN, Alpigiani MG, Rumba I, Sztajnbok F, Tambic-Bukovac L, Breda L, Al-Mayouf S, Mihaylova D, Chasnyk V, Sengler C, Klein-Gitelman M, Djeddi D, Nuno L, Pruunsild C, Brunner J, Kondi A, Pagava K, Pederzoli S, Martini A, Ruperto N. EULAR/PRINTO/PRES criteria for Henoch-Schönlein purpura, childhood polyarteritis nodosa, childhood Wegener

granulomatosis and childhood Takayasu arteritis: Ankara 2008. Part II: Final classification criteria. Ann Rheum Dis. 2010 May;69(5):798-806. eng. Epub 2010/04/24. doi:10.1136/ard.2009.116657. Cited in: Pubmed; PMID 20413568.
80. Mills JA, Michel BA, Bloch DA, Calabrese LH, Hunder GG, Arend WP, Edworthy SM, Fauci AS, Leavitt RY, Lie JT, et al. The American College of Rheumatology 1990 criteria for the classification of Henoch-Schönlein purpura. Arthritis Rheum. 1990 Aug;33(8):1114-21. eng. Epub 1990/08/01. doi:10.1002/art.1780330809. Cited in: Pubmed; PMID 2202310.
81. Hočevar A, Rotar Z, Jurčić V, Pižem J, Čučnik S, Vizjak A, van den Broeke R, Tomšič M. IgA vasculitis in adults: the performance of the EULAR/PRINTO/PRES classification criteria in adults. Arthritis Res Ther. 2016;18:58-58. eng. doi:10.1186/s13075-016-0959-4. Cited in: Pubmed; PMID 26935833.
82. Oni L, Gritzfeld JF, Jones C, Sinha MD, Wallace D, Stack M, Kurt-Sukur ED, Quinlan C, Ruggiero B, Raja M, Tullus K. Comment on: European consensus-based recommendations for diagnosis and treatment of immunoglobulin A vasculitis—the SHARE initiative. Rheumatology. 2021;60(5):e179-e180. doi:10.1093/rheumatology/keaa159.
83. Counahan R, Winterborn MH, White RH, Heaton JM, Meadow SR, Bluett NH, Swetschin H, Cameron JS, Chantler C. Prognosis of Henoch-Schönlein nephritis in children. Br Med J. 1977 Jul 2;2(6078):11-4. eng. Epub 1977/07/02. doi:10.1136/bmj.2.6078.11. Cited in: Pubmed; PMID 871734.
84. Koskela M, Ylinen E, Ukonmaanaho E-M, Autio-Harmainen H, Heikkilä P, Lohi J, Jauhola O, Ronkainen J, Jahnukainen T, Nuutinen M. The ISKDC classification and a new semiquantitative classification for predicting outcomes of Henoch–Schönlein purpura nephritis. Pediatric Nephrology. 2017 2017/07/01;32(7):1201-1209. doi:10.1007/s00467-017-3608-5.
85. Trimarchi H, Barratt J, Cattran DC, Cook HT, Coppo R, Haas M, Liu Z-H, Roberts ISD, Yuzawa Y, Zhang H, Feehally J, Alpers CE, Asunis AM, Barbour S, Becker JU, Ding J, Espino G, Ferrario F, Fogo A, Hladunewich M, Joh K, Katafuchi R, Lv J, Matsuzaki K, Nakanishi K, Pani A, Perera R, Perkowska-Ptasinska A, Reich H, Shima Y, Soares MF, Suzuki Y, Takahashi K, Troyanov S, Verhave JC, Wang S, Weening J, Wyatt R, Yoshikawa N, Zeng C. Oxford Classification of IgA nephropathy 2016: an update from the IgA Nephropathy Classification Working Group. Kidney International. 2017 2017/05/01/;91(5):1014-1021. doi:https://doi.org/10.1016/j.kint.2017.02.003.
86. Akhavan Sepahi M, Mubarak M. Predicting renal outcomes in immunoglobulin A vasculitis nephritis; from ISKDC classification to Oxford MEST-C classification. J Renal Inj Prev. 2020 2020/1/1;9(1):e08-e08. doi:10.15171/jrip.2020.08.
87. Watson L, Richardson ARW, Holt RCL, Jones CA, Beresford MW. Henoch Schonlein Purpura – A 5-Year Review and Proposed Pathway. PLOS ONE. 2012;7(1):e29512. doi:10.1371/journal.pone.0029512.
88. Blanco R, Martínez-Taboada VM, Rodríguez-Valverde V, García-Fuentes M, González-Gay MA. Henoch-Schönlein purpura in adulthood and childhood: two different expressions of the same syndrome. Arthritis Rheum. 1997 May;40(5):859-64. eng. Epub 1997/05/01. doi:10.1002/art.1780400513. Cited in: Pubmed; PMID 9153547.
89. Reamy BV, Servey JT, Williams PM. Henoch-Schönlein Purpura (IgA Vasculitis): Rapid Evidence Review. Am Fam Physician. 2020 Aug 15;102(4):229-233. eng. Epub 2020/08/18. Cited in: Pubmed; PMID 32803924.
90. Prais D, Amir J, Nussinovitch M. Recurrent Henoch-Schönlein Purpura in Children. JCR: Journal of Clinical Rheumatology. 2007;13(1).
91. Fretzayas A, Sionti I, Moustaki M, Papadimitriou A, Nicolaidou P. Henoch-Schönlein purpura: a long-term prospective study in Greek children. J Clin Rheumatol. 2008 Dec;14(6):324-31. eng. Epub 2008/08/16. doi:10.1097/RHU.0b013e31817a240a. Cited in: Pubmed; PMID 18703982.
92. Ekinci RMK, Balci S, Melek E, Karabay Bayazit A, Dogruel D, Altintas DU, Yilmaz M. Clinical manifestations and outcomes of 420 children with Henoch Schönlein Purpura from a single referral center from Turkey: A three-year experience. Modern Rheumatology. 2020 2020/11/01;30(6):1039-1046. doi:10.1080/14397595.2019.1687074.
93. Trapani S, Micheli A, Grisolia F, Resti M, Chiappini E, Falcini F, De Martino M. Henoch Schonlein purpura in childhood: epidemiological and clinical analysis of 150 cases over a 5-year period and review of literature. Semin Arthritis Rheum. 2005 Dec;35(3):143-53. eng. Epub 2005/12/06. doi:10.1016/j.semarthrit.2005.08.007. Cited in: Pubmed; PMID 16325655.
94. Calvo-Río V, Hernández JL, Ortiz-Sanjuán F, Loricera J, Palmou-Fontana N, González-Vela MC, González-Lamuño D, González-López MA, Armesto S, Blanco R, González-Gay MA. Relapses in patients with Henoch-Schönlein purpura: Analysis of 417 patients from a single center. Medicine (Baltimore). 2016;95(28):e4217-e4217. eng. doi:10.1097/MD.0000000000004217. Cited in: Pubmed; PMID 27428226.
95. Gómez S, Pérez M, Pellegrini M, Isern E, Quintana C, Artacho P, Bertolini M, Pomerantz B, Gadda N. Henoch-Schonlein purpura in pediatrics: Ten years of experience at a moderate risk office of a general hospital. Arch Argent Pediatr. 2020 Feb;118(1):31-37. Púrpura de Schonlein-Henoch en pediatría: Diez años de experiencia en un consultorio de moderado riesgo en un hospital general. eng

spa. Epub 2020/01/28. doi:10.5546/aap.2020.eng.31. Cited in: Pubmed; PMID 31984693.
96. Alfredo CS, Nunes NA, Len CA, Barbosa CM, Terreri MT, Hilário MO. Henoch-Schönlein purpura: recurrence and chronicity. J Pediatr (Rio J). 2007 Mar-Apr;83(2):177-80. eng. Epub 2007/03/01. doi:10.2223/jped.1595. Cited in: Pubmed; PMID 17327931.
97. Rigante D, Candelli M, Federico G, Bartolozzi F, Porri MG, Stabile A. Predictive factors of renal involvement or relapsing disease in children with Henoch-Schönlein purpura. Rheumatol Int. 2005 Jan;25(1):45-8. eng. Epub 2004/03/10. doi:10.1007/s00296-004-0452-2. Cited in: Pubmed; PMID 15007622.
98. Zhao YL, Liu ZJ, Bai XM, Wang YC, Li GH, Yan XY. Obesity increases the risk of renal involvement in children with Henoch-Schönlein purpura. Eur J Pediatr. 2015 Oct;174(10):1357-63. eng. Epub 2015/04/23. doi:10.1007/s00431-015-2547-z. Cited in: Pubmed; PMID 25899072.
99. Dundar HA, Pektanc M, Bayram MT, Soylu A, Kavukcu S. Obesity is associated with severe clinical course in children with Henoch-Schonlein purpura. Pediatr Nephrol. 2020 Dec;35(12):2327-2333. eng. Epub 2020/07/03. doi:10.1007/s00467-020-04672-7. Cited in: Pubmed; PMID 32613276.
100. Soylemezoglu O, Ozkaya O, Ozen S, Bakkaloglu A, Dusunsel R, Peru H, Çetinyurek A, Yıldız N, Donmez O, Buyan N, Mir S, Arısoy N, Gur-Guven A, Alpay H, Ekim M, Aksu N, Soylu A, Gok F, Poyrazoglu H, Sonmez F. Henoch-Schönlein Nephritis: A Nationwide Study. Nephron Clinical Practice. 2009;112(3):c199-c204. doi:10.1159/000218109.
101. Tudorache E, Azema C, Hogan J, Wannous H, Aoun B, Decramer S, Deschênes G, Ulinski T. Even mild cases of paediatric Henoch-Schönlein purpura nephritis show significant long-term proteinuria [https://doi.org/10.1111/apa.12723]. Acta Paediatrica. 2015 2015/08/01;104(8):843-848. doi:https://doi.org/10.1111/apa.12723.
102. Ronkainen J, Nuutinen M, Koskimies O. The adult kidney 24 years after childhood Henoch-Schönlein purpura: a retrospective cohort study. The Lancet. 2002;360(9334):666-670. doi:10.1016/S0140-6736(02)09835-5.
103. Butani L, Morgenstern BZ. Long-term Outcome in Children After Henoch-Schönlein Purpura Nephritis. Clinical Pediatrics. 2007 2007/07/01;46(6):505-511. doi:10.1177/0009922806298896.

104. Kawasaki Y, Suyama K, Yugeta E, Katayose M, Suzuki S, Sakuma H, Nemoto K, Tsukagoshi A, Nagasawa K, Hosoya M. The incidence and severity of Henoch-Schönlein purpura nephritis over a 22-year period in Fukushima Prefecture, Japan. International Urology and Nephrology. 2010 2010/12/01;42(4):1023-1029. doi:10.1007/s11255-009-9701-3.
105. Saps M, Dhroove G, Chogle A. Henoch–Schonlein Purpura Leads to Functional Gastrointestinal Disorders. Digestive Diseases and Sciences. 2011 2011/06/01;56(6):1789-1793. doi:10.1007/s10620-010-1499-4.
106. Nikibakhsh AA, Mahmoodzadeh H, Karamyyar M, Hejazi S, Noroozi M, Macooie AA, Gholizadeh A, Gholizadeh L. Treatment of Complicated Henoch-Schönlein Purpura with Mycophenolate Mofetil: A Retrospective Case Series Report. International Journal of Rheumatology. 2010 2010/06/15;2010:254316. doi:10.1155/2010/254316.
107. Donnithorne KJ, Atkinson TP, Hinze CH, Nogueira JB, Saeed SA, Askenazi DJ, Beukelman T, Cron RQ. Rituximab therapy for severe refractory chronic Henoch-Schönlein purpura. J Pediatr. 2009 Jul;155(1):136-9. eng. Epub 2009/06/30. doi:10.1016/j.jpeds.2008.12.049. Cited in: Pubmed; PMID 19559299.
108. Rettig P, Cron RQ. Methotrexate used as a steroid-sparing agent in non-renal chronic Henoch-Schönlein purpura. Clin Exp Rheumatol. 2003 Nov-Dec;21(6):767-9. eng. Epub 2004/01/27. Cited in: Pubmed; PMID 14740458.
109. Crayne CB, Eloseily E, Mannion ML, Azerf SP, Weiser P, Beukelman T, Stoll ML, Feig DI, Prescott Atkinson T, Cron RQ. Rituximab treatment for chronic steroid-dependent Henoch-Schonlein purpura: 8 cases and a review of the literature. Pediatr Rheumatol Online J. 2018 Nov 14;16(1):71. eng. Epub 2018/11/16. doi:10.1186/s12969-018-0285-2. Cited in: Pubmed; PMID 30428889.
110. Chou T, Louissant VR, Adams A, Gurkan S, Chefitz D, Weller AS, Moorthy LN. Successful Treatment of Henoch-Schönlein Purpura With Recurrent Gastrointestinal Involvement With Mycophenolate Mofetil: A Brief Report. Clinical Pediatrics. 2015 2015/08/01;54(9):900-903. doi:10.1177/0009922814568288.
111. Fotis L, Tuttle PVt, Baszis KW, Pepmueller PH, Moore TL, White AJ. Azathioprine therapy for steroid-resistant Henoch-Schönlein purpura: a report of 6 cases. Pediatr Rheumatol Online J. 2016;14(1):37-37. eng. doi:10.1186/s12969-016-0100-x. Cited in: Pubmed; PMID 27333803.
112. Roman C, Dima B, Muyshont L, Schurmans T, Gilliaux O. Indications and efficiency of dapsone in IgA vasculitis (Henoch-Schonlein purpura): case series and a review of the literature. European Journal of Pediatrics. 2019 2019/08/01;178(8):1275-1281. doi:10.1007/s00431-019-03409-5.
113. Chen CB, Garlapati S, Lancaster JD, Zinn Z, Bacaj P, Patra KP. Bullous Henoch-Schönlein purpura in children. Cutis. 2015 Oct;96(4):248-52. eng. Epub 2015/12/19. Cited in: Pubmed; PMID 26682287.
114. Hasbún T, Chaparro X, Kaplan V, Cavagnaro F, Castro A. Bullous Henoch-Schönlein purpura. Case report. Rev Chil Pediatr. 2018 Feb;89(1):103-106. Púrpura de Schönlein-Henoch Buloso. Caso clínico. eng

spa. Epub 2018/04/18. doi:10.4067/s0370-41062018000100103. Cited in: Pubmed; PMID 29664511.
115. Allali S, Fraitag S, Terrier B, Bodemer C, Chalumeau M. Efficacy of colchicine in a child with relapsing bullous Henoch-Schönlein purpura. Eur J Pediatr. 2016 Jan;175(1):147-9. eng. Epub 2015/07/22. doi:10.1007/s00431-015-2594-5. Cited in: Pubmed; PMID 26195178.
116. Saulsbury FT. Successful treatment of prolonged Henoch-Schönlein purpura with colchicine. Clin Pediatr (Phila). 2009 Oct;48(8):866-8. eng. Epub 2009/05/23. doi:10.1177/0009922809337532. Cited in: Pubmed; PMID 19460892.
117. Rovin BH, Adler SG, Barratt J, Bridoux F, Burdge KA, Chan TM, Cook HT, Fervenza FC, Gibson KL, Glassock RJ, Jayne DRW, Jha V, Liew A, Liu Z-H, Mejía-Vilet JM, Nester CM, Radhakrishnan J, Rave EM, Reich HN, Ronco P, Sanders J-SF, Sethi S, Suzuki Y, Tang SCW, Tesar V, Vivarelli M, Wetzels JFM, Floege J. KDIGO 2021 Clinical Practice Guideline for the Management of Glomerular Diseases. Kidney International. 2021;100(4):S1-S276. doi:10.1016/j.kint.2021.05.021.
118. Hahn D, Hodson EM, Willis NS, Craig JC. Interventions for preventing and treating kidney disease in Henoch-Schönlein Purpura (HSP). Cochrane Database Syst Rev. 2015 Aug 7;(8):Cd005128. eng. Epub 2015/08/11. doi:10.1002/14651858.CD005128.pub3. Cited in: Pubmed; PMID 26258874.
119. Berger J, Hinglais N. [Intercapillary deposits of IgA-IgG]. J Urol Nephrol (Paris). 1968 Sep;74(9):694-5. Les ddpôts intercapillaires d'IgA-IgG. fre. Epub 1968/09/01. Cited in: Pubmed; PMID 4180586.
120. Pillebout E. IgA Vasculitis and IgA Nephropathy: Same Disease? J Clin Med. 2021;10(11):2310. eng. doi:10.3390/jcm10112310. Cited in: Pubmed; PMID 34070665.
121. Oni L. The IgA Vasculitis study, study protocol version: 1.3 [unpublished]. 2020 17/12/2020.
122. Aringer M, Costenbader K, Daikh D, Brinks R, Mosca M, Ramsey-Goldman R, Smolen JS, Wofsy D, Boumpas DT, Kamen DL, Jayne D, Cervera R, Costedoat-Chalumeau N, Diamond B, Gladman DD, Hahn B, Hiepe F, Jacobsen S, Khanna D, Lerstrøm K, Massarotti E, McCune J, Ruiz-Irastorza G, Sanchez-Guerrero J, Schneider M, Urowitz M, Bertsias G, Hoyer BF, Leuchten N, Tani C, Tedeschi SK, Touma Z, Schmajuk G, Anic B, Assan F, Chan TM, Clarke AE, Crow MK, Czirják L, Doria A, Graninger W, Halda-Kiss B, Hasni S, Izmirly PM, Jung M, Kumánovics G, Mariette X, Padjen I, Pego-Reigosa JM, Romero-Diaz J, Rúa-Figueroa Fernández Í, Seror R, Stummvoll GH, Tanaka Y, Tektonidou MG, Vasconcelos C, Vital EM, Wallace DJ, Yavuz S, Meroni PL, Fritzler MJ, Naden R, Dörner T, Johnson SR. 2019 European League Against Rheumatism/American College of Rheumatology Classification Criteria for Systemic Lupus Erythematosus. Arthritis Rheumatol. 2019;71(9):1400-1412. eng. Epub 2019/08/06. doi:10.1002/art.40930. Cited in: Pubmed; PMID 31385462.
123. Luca NJ, Feldman BM. Disease activity measures in paediatric rheumatic diseases. International journal of rheumatology. 2013;2013:715352-715352. eng. Epub 2013/09/08. doi:10.1155/2013/715352. Cited in: Pubmed; PMID 24089617.
124. Dolezalova P, Price-Kuehne FE, Özen S, Benseler SM, Cabral DA, Anton J, Brunner J, Cimaz R, O'Neil KM, Wallace CA, Wilkinson N, Eleftheriou D, Demirkaya E, Böhm M, Krol P, Luqmani RA, Brogan PA. Disease activity assessment in childhood vasculitis: development and preliminary validation of the Paediatric Vasculitis Activity Score (PVAS). Annals of the rheumatic diseases. 2012 10/27/entrez

10/27/pubmed

12/16/medline;72(10):1628-33.
125. Luqmani RA, Bacon PA, Moots RJ, Janssen BA, Pall A, Emery P, Savage C, Adu D. Birmingham Vasculitis Activity Score (BVAS) in systemic necrotizing vasculitis. Qjm. 1994 Nov;87(11):671-8. eng. Epub 1994/11/01. Cited in: Pubmed; PMID 7820541.
126. Suzuki H. Biomarkers for IgA nephropathy on the basis of multi-hit pathogenesis. Clinical and experimental nephrology. 2019;23(1):26-31. eng. Epub 2018/05/08. doi:10.1007/s10157-018-1582-2. Cited in: Pubmed; PMID 29740706.
127. Homsak E, Gruson D. Soluble ST2: A complex and diverse role in several diseases. Clin Chim Acta. 2020 Aug;507:75-87. eng. Epub 2020/04/20. doi:10.1016/j.cca.2020.04.011. Cited in: Pubmed; PMID 32305537.
128. Williams CEC, Toner A, Wright RD, Oni L. A systematic review of urine biomarkers in children with IgA vasculitis nephritis. Pediatr Nephrol. 2021;36(10):3033-3044. eng. Epub 2021/05/15. doi:10.1007/s00467-021-05107-7. Cited in: Pubmed; PMID 33993342.

129. Jelusic M, Sestan M, Giani T, Cimaz R. New Insights and Challenges Associated With IgA Vasculitis and IgA Vasculitis With Nephritis-Is It Time to Change the Paradigm of the Most Common Systemic Vasculitis in Childhood? Frontiers in pediatrics. 2022;10:853724-853724. eng. doi:10.3389/fped.2022.853724. Cited in: Pubmed; PMID 35372148.
130. Sestan M, Srsen S, Kifer N, Sapina M, Batnozic Varga M, Ovuka A, Held M, Kozmar A, Frkovic M, Laskarin G, Gagro A, Jelusic M. Persistence and Severity of Cutaneous Manifestations in IgA Vasculitis Is Associated with Development of IgA Vasculitis Nephritis in Children. Dermatology. 2022;238(2):340-346. eng. Epub 2021/06/08. doi:10.1159/000516765. Cited in: Pubmed; PMID 34098552.
131. Williams CEC. Methods of measuring disease activity in paediatric IgA vasculitis: University of Liverpool, Department of Women's and Children's Health; 2021 July 2021. MPhil. https://livrepository.liverpool.ac.uk/3131481/1/201166123_Jul2021.pdf.
132. Collingwood C. Biochemistry Laboratory Handbook. Version 2.10. 2022 28/04/2022. Available from: https://alderhey.nhs.uk/application/files/8516/4762/2842/Alder_Hey_Biochemistry_Laboratory_Handbook_v2.10.pdf.
133. Department of Haematology AHCsNFT. Haematology and Blood Transfusion Laboratory Handbook. Version 1.6. 2022 28/04/2022. Available from: https://alderhey.nhs.uk/application/files/5716/4448/4397/Alder_Hey_Haematology_handbook_2022_v1.6.pdf.
134. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, Shamseer L, Tetzlaff JM, Akl EA, Brennan SE, Chou R, Glanville J, Grimshaw JM, Hróbjartsson A, Lalu MM, Li T, Loder EW, Mayo-Wilson E, McDonald S, McGuinness LA, Stewart LA, Thomas J, Tricco AC, Welch VA, Whiting P, Moher D. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ. 2021;372:n71. doi:10.1136/bmj.n71.
135. Higgins JPT TJ, Chandler J, Cumpston M, Li T, Page MJ, Welch VA (editors). Cochrane Handbook for Systematic Reviews of Interventions version 6.3 (updated February 2022). Version 6.3. Cochrane, editor. 2022. Available from: www.training.cochrane.org/handbook.
136. Oni L. Best practice guideline for the management of complications associated with IgA vasculitis (HSP) in children and young people search strategy [unpublished]. Department of Women's and Children's Health, University of Liverpool. 2021.
137. Ouzzani M, Hammady H, Fedorowicz Z, Elmagarmid A. Rayyan—a web and mobile app for systematic reviews. Systematic Reviews. 2016 2016/12/05;5(1):210. doi:10.1186/s13643-016-0384-4.
138. Programme CAS. CASP Checklists. 2022 30/04/2022. Available from: https://casp-uk.net/casp-tools-checklists/.
139. Gökçe Ş, Kurugöl Z, Koturoğlu G, Aslan A. Predictive role of laboratory markers and clinical features for recurrent Henoch-Schönlein Purpura in childhood: A study from Turkey. Mod Rheumatol. 2020 Nov;30(6):1047-1052. eng. Epub 2019/11/13. doi:10.1080/14397595.2019.1690966. Cited in: Pubmed; PMID 31711347.
140. Fan GZ, Li RX, Jiang Q, Niu MM, Qiu Z, Chen WX, Liu HH, Ruan JW, Hu P. Streptococcal infection in childhood Henoch-Schönlein purpura: a 5-year retrospective study from a single tertiary medical center in China, 2015-2019. Pediatr Rheumatol Online J. 2021;19(1):79-79. eng. doi:10.1186/s12969-021-00569-3. Cited in: Pubmed; PMID 34078391.
141. Teng M-C, Wang L-C, Yu H-H, Lee J-H, Yang Y-H, Chiang B-L. Kawasaki disease and Henoch–Schönlein purpura – 10 years' experience of childhood vasculitis at a university hospital in Taiwan. Journal of Microbiology, Immunology and Infection. 2012 2012/02/01/;45(1):22-30. doi:https://doi.org/10.1016/j.jmii.2011.09.024.
142. Farisogullari B, Cuceoglu MK, Oral H, Yardimci GK, Bilginer Y, Ozen S, Karadag O. Biologics for immunoglobulin A vasculitis: targeting vasculitis or comorbid disease? Internal and Emergency Medicine. 2022 2022/03/26. doi:10.1007/s11739-022-02968-0.
143. Schinzel V, Fernandez JD, Clemente G, Fraga MM, Andrade MC, Len CA, Terreri MT. The profile and clinical outcomes of patients with renal involvement due to IgA vasculitis: is azathioprine a good option for treatment? Advances in Rheumatology. 2019 2019/05/22;59(1):21. doi:10.1186/s42358-019-0064-x.
144. Buscatti IM, Casella BB, Aikawa NE, Watanabe A, Farhat SCL, Campos LMA, Silva CA. Henoch-Schönlein purpura nephritis: initial risk factors and outcomes in a Latin American tertiary center. Clinical Rheumatology. 2018 2018/05/01;37(5):1319-1324. doi:10.1007/s10067-017-3972-3.
145. de Almeida JL, Campos LM, Paim LB, Leone C, Koch VH, Silva CA. Renal involvement in Henoch-Schönlein purpura: a multivariate analysis of initial prognostic factors. J Pediatr (Rio J). 2007 May-Jun;83(3):259-66. eng. Epub 2007/06/07. doi:10.2223/jped.1638. Cited in: Pubmed; PMID 17551657.
146. Sano H, Izumida M, Shimizu H, Ogawa Y. Risk factors of renal involvement and significant proteinuria in Henoch-Schönlein purpura. European Journal of Pediatrics. 2002 2002/04/01;161(4):196-201. doi:10.1007/s00431-002-0922-z.
147. Luciana B, Ilaria C, Isabella C, Gentile C, Grasso EA, Di Donato G, Francesco C, Verrotti A. Epidemiological and clinical aspects of immunoglobulin A vasculitis in childhood: a retrospective cohort study. Italian Journal of Pediatrics. 2021 2021/12/15;47(1):237. doi:10.1186/s13052-021-01182-6.
148. Wananukul S, Voramethkul W, Kaewopas Y, Hanvivatvong O. Prevalence of positive antinuclear antibodies in healthy children. Asian Pac J Allergy Immunol. 2005 Jun-Sep;23(2-3):153-7. eng. Epub 2005/10/29. Cited in: Pubmed; PMID 16252846.
149. Hilário MO, Len CA, Roja SC, Terreri MT, Almeida G, Andrade LE. Frequency of antinuclear antibodies in healthy children and adolescents. Clin Pediatr (Phila). 2004 Sep;43(7):637-42. eng. Epub 2004/09/21. doi:10.1177/000992280404300709. Cited in: Pubmed; PMID 15378151.
150. Yin DH, Guo YL, Cao TT, Pan CL, Zhao GJ, Hu Y. Effect of animal protein diet on the prognosis of children with Henoch-Schönlein purpura. Zhongguo Dang Dai Er Ke Za Zhi. 2021 Sept 15;23(9):927-932. 动物蛋白饮食对过敏性紫癜患儿预后的影响分析. eng chi. Epub 2021/09/19. doi:10.7499/j.issn.1008-8830.2106126. Cited in: Pubmed; PMID 34535208.
151. Weiss PF, Klink AJ, Hexem K, Burnham JM, Leonard MB, Keren R, Localio R, Feudtner C. Variation in inpatient therapy and diagnostic evaluation of children with Henoch Schönlein purpura. The Journal of pediatrics. 2009;155(6):812-818.e1. eng. Epub 2009/07/29. doi:10.1016/j.jpeds.2009.05.030. Cited in: Pubmed; PMID 19643437.
152. Ronkainen J, Koskimies O, Ala-Houhala M, Antikainen M, Merenmies J, Rajantie J, Örmälä T, Turtinen J, Nuutinen M. Early prednisone therapy in Henoch-Schönlein purpura: A randomized, double-blind, placebo-controlled trial. The Journal of Pediatrics. 2006 2006/08/01/;149(2):241-247. doi:https://doi.org/10.1016/j.jpeds.2006.03.024.
153. Weiss PF, Klink AJ, Localio R, Hall M, Hexem K, Burnham JM, Keren R, Feudtner C. Corticosteroids may improve clinical outcomes during hospitalization for Henoch-Schönlein purpura. Pediatrics. 2010 Oct;126(4):674-81. eng. Epub 2010/09/22. doi:10.1542/peds.2009-3348. Cited in: Pubmed; PMID 20855386.
154. Kocaoglu C, Ozturk R, Unlu Y, Akyurek FT, Arslan S. Successful Treatment of Hemorrhagic Bullous Henoch-Schönlein Purpura with Oral Corticosteroid: A Case Report. Case Reports in Pediatrics. 2013 2013/04/16;2013:680208. doi:10.1155/2013/680208.
155. Aljebab F, Choonara I, Conroy S. Systematic Review of the Toxicity of Long-Course Oral Corticosteroids in Children. PloS one. 2017;12(1):e0170259-e0170259. eng. doi:10.1371/journal.pone.0170259. Cited in: Pubmed; PMID 28125632.
156. Morris PJ. Chapter 15 - Azathioprine. In: Morris PJ, Knechtle SJ, editors. Kidney Transplantation–Principles and Practice (Seventh Edition). Philadelphia (PA): W.B. Saunders; 2014. p. 216-220.

157. Carlson JA, Cavaliere LF, Grant-Kels JM. Cutaneous vasculitis: diagnosis and management. Clinics in Dermatology. 2006 2006/09/01/;24(5):414-429. doi:https://doi.org/10.1016/j.clindermatol.2006.07.007.
158. Goeser MR, Laniosz V, Wetter DA. A Practical Approach to the Diagnosis, Evaluation, and Management of Cutaneous Small-Vessel Vasculitis. American Journal of Clinical Dermatology. 2014 2014/08/01;15(4):299-306. doi:10.1007/s40257-014-0076-6.
159. Lee KH, Hong SH, Jun J, Jo Y, Jo W, Choi D, Joo J, Jung G, Ahn S, Kronbichler A, Eisenhut M, Shin JI. Treatment of refractory IgA vasculitis with dapsone: a systematic review. Clin Exp Pediatr. 2020 May;63(5):158-163. eng. Epub 2020/02/07. doi:10.3345/kjp.2019.00514. Cited in: Pubmed; PMID 32024340.
160. Micheletti RG, Pagnoux C, Tamura RN, Grayson PC, McAlear CA, Borchin R, Krischer JP, Merkel PA, Vasculitis Clinical Research C. Protocol for a randomized multicenter study for isolated skin vasculitis (ARAMIS) comparing the efficacy of three drugs: azathioprine, colchicine, and dapsone. Trials. 2020;21(1):362-362. eng. doi:10.1186/s13063-020-04285-3. Cited in: Pubmed; PMID 32345372.
161. Casian A, Sangle SR, D'Cruz DP. New use for an old treatment: Hydroxychloroquine as a potential treatment for systemic vasculitis. Autoimmunity Reviews. 2018 2018/07/01/;17(7):660-664. doi:https://doi.org/10.1016/j.autrev.2018.01.016.
162. Van de Perre E, Jones RB, Jayne DRW. IgA vasculitis (Henoch–Schönlein purpura): refractory and relapsing disease course in the adult population. Clinical Kidney Journal. 2021;14(8):1953-1960. doi:10.1093/ckj/sfaa251.
163. Kusuda A, Migita K, Tsuboi M, Degawa M, Matsuoka N, Tominaga M, Kawakami A, Kawabe Y, Taguchi T, Eguchi K. Successful treatment of adult-onset Henoch-Schönlein purpura nephritis with high-dose immunoglobulins. Intern Med. 1999 Apr;38(4):376-9. eng. Epub 1999/06/11. doi:10.2169/internalmedicine.38.376. Cited in: Pubmed; PMID 10361914.
164. Ruellan A, Khatibi M, Staub T, Martin T, Storck D, Christmann D. Purpura rhumatoïde et immunoglobulines intraveineuses. La Revue de Médecine Interne. 1997 1997/09/01/;18(9):727-729. doi:https://doi.org/10.1016/S0248-8663(97)83754-X.
165. Heldrich FJ, Minkin S, Gatdula CL. Intravenous immunoglobulin in Henoch-Schönlein purpura: a case study. Md Med J. 1993 1993/06//;42(6):577-579. eng. Cited in: Pubmed; PMID 8377631.
166. Morotti F, Bracciolini G, Caorsi R, Cattaneo L, Gattorno M, Ravelli A, Felici E. Intravenous immunoglobulin for corticosteroid-resistant intestinal Henoch-Schönlein purpura: worth a controlled trial against corticosteroids? Rheumatology. 2021;60(8):3868-3871. doi:10.1093/rheumatology/keaa743.
167. Hernández-Rodríguez J, Carbonell C, Mirón-Canelo JA, Diez-Ruiz S, Marcos M, Chamorro AJ. Rituximab treatment for IgA vasculitis: A systematic review. Autoimmun Rev. 2020 Apr;19(4):102490. eng. Epub 2020/02/18. doi:10.1016/j.autrev.2020.102490. Cited in: Pubmed; PMID 32062030.
168. Miray Kisla Ekinci R, Balci S, Serbes M, Duyuler Ayçin G, Dogruel D, Ufuk Altintas D, Yilmaz M. Recurrent Henoch Schönlein purpura without renal involvement successfully treated with methotrexate. Scott Med J. 2019 May;64(2):74-77. eng. Epub 2018/11/21. doi:10.1177/0036933018809816. Cited in: Pubmed; PMID 30453828.
169. Inoue CN, Nagasaka T, Matsutani S, Ishidoya M, Homma R, Chiba Y. Efficacy of early dental and ENT therapy in preventing nephropathy in pediatric Henoch-Schönlein purpura. Clin Rheumatol. 2008 Dec;27(12):1489-96. eng. Epub 2008/07/25. doi:10.1007/s10067-008-0954-5. Cited in: Pubmed; PMID 18651102.
170. Inoue CN, Chiba Y, Morimoto T, Nishio T, Kondo Y, Adachi M, Matsutani S. Tonsillectomy in the treatment of pediatric Henoch-Schönlein nephritis. Clin Nephrol. 2007 May;67(5):298-305. eng. Epub 2007/06/05. doi:10.5414/cnp67298. Cited in: Pubmed; PMID 17542339.
171. Ohara S, Kawasaki Y, Matsuura H, Oikawa T, Suyama K, Hosoya M. Successful therapy with tonsillectomy for severe ISKDC grade VI Henoch-Schönlein purpura nephritis and persistent nephrotic syndrome. Clin Exp Nephrol. 2011 Oct;15(5):749-753. eng. Epub 2011/05/28. doi:10.1007/s10157-011-0463-8. Cited in: Pubmed; PMID 21618075.
172. Kanai H, Sawanobori E, Kobayashi A, Matsushita K, Sugita K, Higashida K. Early treatment with methylprednisolone pulse therapy combined with tonsillectomy for heavy proteinuric henoch-schönlein purpura nephritis in children. Nephron Extra. 2011 Jan;1(1):101-11. eng. Epub 2011/01/01. doi:10.1159/000333010. Cited in: Pubmed; PMID 22470384.
173. Feehally J, Coppo R, Troyanov S, Bellur SS, Cattran D, Cook T, Roberts IS, Verhave JC, Camilla R, Vergano L, Egido J, Wiecek A, Karkoszka H, Tesar V, Maixnerova D, Ots-Rosenberg M, Quaglia M, Rollino C, Magistroni R, Cusinato S, Cravero R, Peruzzi L, Lundberg S, Gesualdo L, Cancarini G, Feriozzi S, Ferrario F. Tonsillectomy in a European Cohort of 1,147 Patients with IgA Nephropathy. Nephron. 2016;132(1):15-24. eng. Epub 2015/11/21. doi:10.1159/000441852. Cited in: Pubmed; PMID 26586175.
174. Demirkaya E, Ozen S, Pistorio A, Galasso R, Ravelli A, Hasija R, Baskin E, Dressler F, Fischbach M, Garcìa Consuegra J, Iagaru N, Pasic S, Scarpato S, van Rossum MA, Apaz MT, Barash J, Calcagno G, Gonzalez B, Hoppenreijs E, Ioseliani M, Mazur-Zielinska H, Vougiouka O, Wulffraat N, Luqmani R, Martini A, Ruperto N, Dolezalova P. Performance of Birmingham Vasculitis Activity Score and disease extent index in childhood vasculitides. Clin Exp Rheumatol. 2012 Jan-Feb;30(1 Suppl 70):S162-8. eng. Epub 2012/07/26. Cited in: Pubmed; PMID 22640658.
175. Charles P, Terrier B, Perrodeau É, Cohen P, Faguer S, Huart A, Hamidou M, Agard C, Bonnotte B, Samson M, Karras A, Jourde-Chiche N, Lifermann F, Gobert P, Hanrotel-Saliou C, Godmer P, Martin-Silva N, Pugnet G, Matignon M, Aumaitre O, Viallard JF, Maurier F, Meaux-Ruault N, Rivière S, Sibilia J, Puéchal X, Ravaud P, Mouthon L, Guillevin L. Comparison of individually tailored versus fixed-schedule rituximab regimen to maintain ANCA-associated vasculitis remission: results of a multicentre, randomised controlled, phase III trial (MAINRITSAN2). Ann Rheum Dis. 2018 Aug;77(8):1143-1149. eng. Epub 2018/04/27. doi:10.1136/annrheumdis-2017-212878. Cited in: Pubmed; PMID 29695500.
176. Gopaluni S, Smith R, Goymer D, Cahill H, Broadhurst E, Wallin E, McClure M, Chaudhry A, Jayne D. Alemtuzumab for refractory primary systemic vasculitis-a randomised controlled dose ranging clinical trial of efficacy and safety (ALEVIATE). Arthritis Res Ther. 2022;24(1):81-81. eng. doi:10.1186/s13075-022-02761-6. Cited in: Pubmed; PMID 35365179.
177. Szpirt WM, Heaf JG, Petersen J. Plasma exchange for induction and cyclosporine A for maintenance of remission in Wegener's granulomatosis—a clinical randomized controlled trial. Nephrology Dialysis Transplantation. 2011;26(1):206-213. doi:10.1093/ndt/gfq360.
178. Sanders J-SF, de Joode AAE, DeSevaux RG, Broekroelofs J, Voskuyl AE, van Paassen P, Kallenberg CGM, Tervaert JWC, Stegeman CA. Extended versus standard azathioprine maintenance therapy in newly diagnosed proteinase-3 anti-neutrophil cytoplasmic antibody-associated vasculitis patients who remain cytoplasmic anti-neutrophil cytoplasmic antibody-positive after induction of remission: a randomized clinical trial. Nephrology Dialysis Transplantation. 2016;31(9):1453-1459. doi:10.1093/ndt/gfw211.
179. Jayne DRW, Merkel PA, Schall TJ, Bekker P. Avacopan for the Treatment of ANCA-Associated Vasculitis. New England Journal of Medicine. 2021 2021/02/18;384(7):599-609. doi:10.1056/NEJMoa2023386.
180. Hellmich B, Flossmann O, Gross WL, Bacon P, Willem Cohen-Tervaert J, Guillevin L, Jayne D, Mahr A, Merkel PA, Raspe H, Scott DGI, Witter J, Yazici H, Luqmani RA. EULAR recommendations for conducting clinical studies and/or clinical trials in systemic vasculitis: focus on anti-neutrophil cytoplasm antibody-associated vasculitis. Annals of the Rheumatic Diseases. 2007;66(5):605. doi:10.1136/ard.2006.062711.
181. Lee J-S, Kim J-G, Lee S. Clinical presentations and long term prognosis of childhood onset polyarteritis nodosa in single centre of Korea. Scientific Reports. 2021 2021/04/16;11(1):8393. doi:10.1038/s41598-021-87718-6.

182. Eleftheriou D, Gale H, Pilkington C, Fenton M, Sebire NJ, Brogan PA. Eosinophilic granulomatosis with polyangiitis in childhood: retrospective experience from a tertiary referral centre in the UK. Rheumatology. 2016;55(7):1263-1272. doi:10.1093/rheumatology/kew029.
183. Luqmani RA. Disease assessment in systemic vasculitis. Nephrology Dialysis Transplantation. 2015;30(suppl_1):i76-i82. doi:10.1093/ndt/gfv002.
184. Morishita KA, Moorthy LN, Lubieniecka JM, Twilt M, Yeung RSM, Toth MB, Shenoi S, Ristic G, Nielsen SM, Luqmani RA, Li SC, Lee T, Lawson EF, Kostik MM, Klein-Gitelman M, Huber AM, Hersh AO, Foell D, Elder ME, Eberhard BA, Dancey P, Charuvanij S, Benseler SM, Cabral DA, for the AINwtPI. Early Outcomes in Children With Antineutrophil Cytoplasmic Antibody–Associated Vasculitis [https://doi.org/10.1002/art.40112]. Arthritis & Rheumatology. 2017 2017/07/01;69(7):1470-1479. doi:https://doi.org/10.1002/art.40112.
185. Anil M, Aksu N, Kara OD, Bal A, Anil AB, Yavaşcan O, Un B. Henoch-Schönlein purpura in children from western Turkey: a retrospective analysis of 430 cases. Turk J Pediatr. 2009 Sep-Oct;51(5):429-36. eng. Epub 2010/02/02. Cited in: Pubmed; PMID 20112597.
186. De Vere Hunt IJ, Howard E, McPherson T. The impact of chronic skin disease in adolescence and the need for specialist adolescent services. Clinical and Experimental Dermatology. 2020;45(1):5-9. doi:https://doi.org/10.1111/ced.14021.
187. Yaxing L, Xia S, Shu L, Zhihong L, Qiaoju Y, Yanwei W, Tianxue G, Jun Z. Parents Resilience Affects Condition of Children With Henoch-Schonlein Purpura Nephritis. The Journal for Nurse Practitioners. 2019;15(10):796-800. doi:10.1016/j.nurpra.2019.08.014.
188. Cheung CK, Rajasekaran A, Barratt J, Rizk DV. An Update on the Current State of Management and Clinical Trials for IgA Nephropathy. J Clin Med. 2021;10(11). doi:10.3390/jcm10112493.
189. Dumont C, Mérouani A, Ducruet T, Benoit G, Clermont M-J, Lapeyraque AL, Phan V, Patey N. Clinical relevance of membrane attack complex deposition in children with IgA nephropathy and Henoch-Schönlein purpura. Pediatric Nephrology. 2020 2020/05/01;35(5):843-850. doi:10.1007/s00467-019-04445-x.
190. Lurbe E, Agabiti-Rosei E, Cruickshank JK, Dominiczak A, Erdine S, Hirth A, Invitti C, Litwin M, Mancia G, Pall D, Rascher W, Redon J, Schaefer F, Seeman T, Sinha M, Stabouli S, Webb NJ, Wühl E, Zanchetti A. 2016 European Society of Hypertension guidelines for the management of high blood pressure in children and adolescents. J Hypertens. 2016 Oct;34(10):1887-920. eng. Epub 2016/07/29. doi:10.1097/hjh.0000000000001039. Cited in: Pubmed; PMID 27467768.
191. Asano K, Iwasaki H, Fujimura K, Ikeda M, Sugimoto Y, Matsubara A, Yano K, Irisawa H, Kono F, Kanbe M, et al. Automated microanalysis of creatinine by coupled enzyme reactions. Hiroshima J Med Sci. 1992 Mar;41(1):1-5. eng. Epub 1992/03/01. Cited in: Pubmed; PMID 1572805.
192. Ogden TL. Handling results below the level of detection. The Annals of Occupational Hygiene. 2010;54(3):255-256. doi:10.1093/annhyg/mep099.
193. Mukaka MM. Statistics corner: A guide to appropriate use of correlation coefficient in medical research. Malawi Med J. 2012;24(3):69-71. eng. Cited in: Pubmed; PMID 23638278.
194. Mandrekar JN. Receiver Operating Characteristic Curve in Diagnostic Test Assessment. Journal of Thoracic Oncology. 2010 2010/09/01/;5(9):1315-1316. doi:https://doi.org/10.1097/JTO.0b013e3181ec173d.
195. Pillebout E, Jamin A, Ayari H, Housset P, Pierre M, Sauvaget V, Viglietti D, Deschenes G, Monteiro RC, Berthelot L, group HS. Biomarkers of IgA vasculitis nephritis in children. PloS one. 2017;12(11):e0188718-e0188718. eng. doi:10.1371/journal.pone.0188718. Cited in: Pubmed; PMID 29190714.
196. Berthelot L, Jamin A, Viglietti D, Chemouny JM, Ayari H, Pierre M, Housset P, Sauvaget V, Hurtado-Nedelec M, Vrtovsnik F, Daugas E, Monteiro RC, Pillebout E. Value of biomarkers for predicting immunoglobulin A vasculitis nephritis outcome in an adult prospective cohort. Nephrol Dial Transplant. 2018 Sep 1;33(9):1579-1590. eng. Epub 2017/11/11. doi:10.1093/ndt/gfx300. Cited in: Pubmed; PMID 29126311.
197. Kang ZJ, Liu B, Li ZH, Duan CR, Wu TH, Xun M, Zhang Y, Ding YF, Fu RQ. [Value of galactose-deficient IgA1 in the early diagnosis of Henoch-Schönlein purpura nephritis in children]. Zhongguo Dang Dai Er Ke Za Zhi. 2019 Feb;21(2):172-175. chi. Epub 2019/02/21. doi:10.7499/j.issn.1008-8830.2019.02.013. Cited in: Pubmed; PMID 30782282.
198. Suzuki H, Allegri L, Suzuki Y, Hall S, Moldoveanu Z, Wyatt RJ, Novak J, Julian BA. Galactose-Deficient IgA1 as a Candidate Urinary Polypeptide Marker of IgA Nephropathy? Disease markers. 2016;2016:7806438-7806438. eng. Epub 2016/08/28. doi:10.1155/2016/7806438. Cited in: Pubmed; PMID 27647947.
199. Onishi Y, Mise K, Kawakita C, Uchida HA, Sugiyama H, Sugawara R, Yamaguchi S, Yoshida M, Mitsuhashi T, Yamada M, Hirabayashi J, Wada J. Development of Urinary Diagnostic Biomarker for IgA Nephropathy by Lectin Microarray. American Journal of Nephrology. 2022;53(1):10-20. doi:10.1159/000520998.
200. Matousovic K, Novak J, Yanagihara T, Tomana M, Moldoveanu Z, Kulhavy R, Julian BA, Konecny K, Mestecky J. IgA-containing immune complexes in the urine of IgA nephropathy patients. Nephrol Dial Transplant. 2006 Sep;21(9):2478-84. eng. Epub 2006/06/08. doi:10.1093/ndt/gfl240. Cited in: Pubmed; PMID 16757497.
201. Calvo-Río V, Loricera J, Mata C, Martín L, Ortiz-Sanjuán F, Alvarez L, González-Vela MC, González-Lamuño D, Rueda-Gotor J, Fernández-Llaca H, González-López MA, Armesto S, Peiró E, Arias M, González-Gay MA, Blanco R. Henoch-Schönlein purpura in northern Spain: clinical spectrum of the disease in 417 patients from a single center. Medicine (Baltimore). 2014;93(2):106-113. eng. doi:10.1097/MD.0000000000000019. Cited in: Pubmed; PMID 24646467.
202. Xi L, Xu S, Jiang Y, Su H, Sun Y, Wen Y, Wu J, Ren X. Predictive Value of Laboratory Indexes on Renal Involvement in Children with Henoch-Schönlein Purpura. Int J Gen Med. 2021;14:7951-7959. eng. Epub 2021/11/20. doi:10.2147/ijgm.S337437. Cited in: Pubmed; PMID 34795511.
203. Galla JH, Spotswood MF, Harrison LA, Mestecky J. Urinary IgA in IgA nephropathy and Henoch-Schoenlein purpura. J Clin Immunol. 1985 Sep;5(5):298-306. eng. Epub 1985/09/01. doi:10.1007/bf00918248. Cited in: Pubmed; PMID 4055989.
204. Shin JI, Park JM, Shin YH, Lee JS, Jeong HJ, Kim HS. Serum IgA/C3 ratio may be a useful marker of disease activity in severe Henoch-Schönlein nephritis. Nephron Clin Pract. 2005;101(2):c72-8. eng. Epub 2005/06/09. doi:10.1159/000086225. Cited in: Pubmed; PMID 15942254.
205. Allen AC, Willis FR, Beattie TJ, Feehally J. Abnormal IgA glycosylation in Henoch-Schönlein purpura restricted to patients with clinical nephritis. Nephrol Dial Transplant. 1998 Apr;13(4):930-4. eng. Epub 1998/05/06. doi:10.1093/ndt/13.4.930. Cited in: Pubmed; PMID 9568852.
206. Lau KK, Wyatt RJ, Moldoveanu Z, Tomana M, Julian BA, Hogg RJ, Lee JY, Huang WQ, Mestecky J, Novak J. Serum levels of galactose-deficient IgA in children with IgA nephropathy and Henoch-Schönlein purpura. Pediatr Nephrol. 2007 Dec;22(12):2067-72. eng. Epub 2007/10/19. doi:10.1007/s00467-007-0623-y. Cited in: Pubmed; PMID 17943324.
207. Tang M, Zhang X, Li X, Lei L, Zhang H, Ling C, Ni J, Lv J, Liu X, Chen X. Serum levels of galactose-deficient IgA1 in Chinese children with IgA nephropathy, IgA vasculitis with nephritis, and IgA vasculitis. Clin Exp Nephrol. 2021 Jan;25(1):37-43. eng. Epub 2020/09/17. doi:10.1007/s10157-020-01968-8. Cited in: Pubmed; PMID 32935202.
208. Suzuki H, Moldoveanu Z, Julian BA, Wyatt RJ, Novak J. Autoantibodies Specific for Galactose-Deficient IgA1 in IgA Vasculitis With Nephritis. Kidney Int Rep. 2019 Dec;4(12):1717-1724. eng. Epub 2019/12/18. doi:10.1016/j.ekir.2019.08.015. Cited in: Pubmed; PMID 31844808.

209. Lacerda Mariano L, Ingersoll MA. The immune response to infection in the bladder. Nature Reviews Urology. 2020 2020/08/01;17(8):439-458. doi:10.1038/s41585-020-0350-8.
210. James-Ellison MY, Roberts R, Verrier-Jones K, Williams JD, Topley N. Mucosal immunity in the urinary tract: changes in sIgA, FSC and total IgA with age and in urinary tract infection. Clin Nephrol. 1997 Aug;48(2):69-78. eng. Epub 1997/08/01. Cited in: Pubmed; PMID 9285142.
211. Paar M, Fengler VH, Rosenberg DJ, Krebs A, Stauber RE, Oettl K, Hammel M. Albumin in patients with liver disease shows an altered conformation. Commun Biol. 2021;4(1):731-731. eng. doi:10.1038/s42003-021-02269-w. Cited in: Pubmed; PMID 34127764.
212. Schenk S, Schoenhals GJ, de Souza G, Mann M. A high confidence, manually validated human blood plasma protein reference set. BMC Med Genomics. 2008;1:41-41. eng. doi:10.1186/1755-8794-1-41. Cited in: Pubmed; PMID 18793429.
213. Torun Bayram M, Heybeli C, Yıldız G, Soylu A, Celik A, Sarioglu S, Kavukçu S. Comparison of clinical, pathological and long-term renal outcomes of children with Henoch–Schonlein purpura nephritis and IgA nephropathy. International Urology and Nephrology. 2021 2021/11/30. doi:10.1007/s11255-021-03063-7.
214. Söylemezoglu O, Sultan N, Gursel T, Buyan N, Hasanoglu E. Circulating adhesion molecules ICAM-1, E-selectin, and von Willebrand factor in Henoch-Schönlein purpura. Arch Dis Child. 1996 Dec;75(6):507-11. eng. Epub 1996/12/01. doi:10.1136/adc.75.6.507. Cited in: Pubmed; PMID 9014604.
215. Mrowka C, Heintz B, Sieberth HG. VCAM-1, ICAM-1, and E-selectin in IgA nephropathy and Schönlein-Henoch syndrome: differences between tissue expression and serum concentration. Nephron. 1999;81(3):256-63. eng. Epub 1999/03/02. doi:10.1159/000045290. Cited in: Pubmed; PMID 10050078.
216. Wang Z, Xie X, Li J, Zhang X, He J, Wang M, Lv J, Zhang H. Complement Activation Is Associated With Crescents in IgA Nephropathy. Front Immunol. 2021;12:676919-676919. eng. doi:10.3389/fimmu.2021.676919. Cited in: Pubmed; PMID 34594322.
217. Genest DS, Bonnefoy A, Khalili M, Merlen C, Genest G, Lapeyraque A-L, Patey N, Smail N, Royal V, Troyanov S. Comparison of Complement Pathway Activation in Autoimmune Glomerulonephritis. Kidney Int Rep. 2022;7(5):1027-1036. eng. doi:10.1016/j.ekir.2022.02.002. Cited in: Pubmed; PMID 35571000.
218. Liu L, Zhang Y, Duan X, Peng Q, Liu Q, Zhou Y, Quan S, Xing G. C3a, C5a renal expression and their receptors are correlated to severity of IgA nephropathy. J Clin Immunol. 2014 Feb;34(2):224-32. eng. Epub 2013/12/12. doi:10.1007/s10875-013-9970-6. Cited in: Pubmed; PMID 24327134.
219. Selvaskandan H, Kay Cheung C, Dormer J, Wimbury D, Martinez M, Xu G, Barratt J. Inhibition of the Lectin Pathway of the Complement System as a Novel Approach in the Management of IgA Vasculitis-Associated Nephritis. Nephron. 2020;144(9):453-458. doi:10.1159/000508841.
220. Lee A. Avacopan: First Approval. Drugs. 2022 2022/01/01;82(1):79-85. doi:10.1007/s40265-021-01643-6.
221. Bruchfeld A, Magin H, Nachman P, Parikh S, Lafayette R, Potarca A, Miao S, Bekker P. C5a receptor inhibitor avacopan in immunoglobulin A nephropathy-an open-label pilot study. Clinical kidney journal. 2022;15(5):922-928. eng. doi:10.1093/ckj/sfab294. Cited in: Pubmed; PMID 35498891.
222. Davin J-C, Coppo R. Henoch–Schönlein purpura nephritis in children. Nature Reviews Nephrology. 2014 2014/10/01;10(10):563-573. doi:10.1038/nrneph.2014.126.
223. Ozen S, Pistorio A, Iusan SM, Bakkaloglu A, Herlin T, Brik R, Buoncompagni A, Lazar C, Bilge I, Uziel Y, Rigante D, Cantarini L, Hilario MO, Silva CA, Alegria M, Norambuena X, Belot A, Berkun Y, Estrella AI, Olivieri AN, Alpigiani MG, Rumba I, Sztajnbok F, Tambic-Bukovac L, Breda L, Al-Mayouf S, Mihaylova D, Chasnyk V, Sengler C, Klein-Gitelman M, Djeddi D, Nuno L, Pruunsild C, Brunner J, Kondi A, Pagava K, Pederzoli S, Martini A, Ruperto N, for the Paediatric Rheumatology International Trials O. EULAR/PRINTO/PRES criteria for Henoch–Schönlein purpura, childhood polyarteritis nodosa, childhood Wegener granulomatosis and childhood Takayasu arteritis: Ankara 2008. Part II: Final classification criteria. Annals of the Rheumatic Diseases. 2010;69(5):798. doi:10.1136/ard.2009.116657.
224. Watson L, Richardson AR, Holt RC, Jones CA, Beresford MW. Henoch schonlein purpura--a 5-year review and proposed pathway. PLoS One. 2012;7(1):e29512. eng. Epub 2012/01/12. doi:10.1371/journal.pone.0029512. Cited in: Pubmed; PMID 22235302.
225. Schneider CA, Rasband WS, Eliceiri KW. NIH Image to ImageJ: 25 years of image analysis. Nature Methods. 2012 2012/07/01;9(7):671-675. doi:10.1038/nmeth.2089.
226. Carpentier G, Henault E. Protein array analyzer for ImageJ. ImageJ User and Developer Conference; 2010. 238-240 p.
227. Pang Z, Chong J, Zhou G, de Lima Morais DA, Chang L, Barrette M, Gauthier C, Jacques P-É, Li S, Xia J. MetaboAnalyst 5.0: narrowing the gap between raw spectra and functional insights. Nucleic Acids Research. 2021;49(W1):W388-W396. doi:10.1093/nar/gkab382.
228. Blighe K, S Rana, and M Lewis. EnhancedVolcano: Publication-ready volcano plots with enhanced colouring and labeling. 2018.
229. Moisini I, Davidson A. BAFF: a local and systemic target in autoimmune diseases. Clin Exp Immunol. 2009 Nov;158(2):155-63. eng. Epub 2009/09/10. doi:10.1111/j.1365-2249.2009.04007.x. Cited in: Pubmed; PMID 19737141.
230. Prieto-Peña D, Genre F, Remuzgo-Martínez S, Pulito-Cueto V, Atienza-Mateo B, Llorca J, Sevilla-Pérez B, Ortego-Centeno N, Lera-Gómez L, Leonardo MT, Peñalba A, Narváez J, Martín-Penagos L, Rodrigo E, Miranda-Filloy JA, Caminal-Montero L, Collado P, Pérez JS, de Argila D, Rubio E, Luque ML, Blanco-Madrigal JM, Galíndez-Agirregoikoa E, Gualillo O, Martín J, Castañeda S, Blanco R, González-Gay MA, López-Mejías R. BAFF, APRIL and BAFFR on the pathogenesis of Immunoglobulin-A vasculitis. Scientific Reports. 2021 2021/06/01;11(1):11510. doi:10.1038/s41598-021-91055-z.
231. Cao Y, Lu G, Chen X, Chen X, Guo N, Li W. BAFF is involved in the pathogenesis of IgA nephropathy by activating the TRAF6/NF-κB signaling pathway in glomerular mesangial cells. Mol Med Rep. 2020 Feb;21(2):795-805. eng. Epub 2020/01/25. doi:10.3892/mmr.2019.10870. Cited in: Pubmed; PMID 31974601.
232. Huang X, Xu G. An Update on Targeted Treatment of IgA Nephropathy: An Autoimmune Perspective [Review]. Frontiers in Pharmacology. 2021 2021-August-23;12. English. doi:10.3389/fphar.2021.715253.
233. Li W, Peng X, Liu Y, Liu H, Liu F, He L, Liu Y, Zhang F, Guo C, Chen G, Zhang L, Dong Z, Peng Y. TLR9 and BAFF: their expression in patients with IgA nephropathy. Mol Med Rep. 2014 Sep;10(3):1469-74. eng. Epub 2014/07/06. doi:10.3892/mmr.2014.2359. Cited in: Pubmed; PMID 24993857.
234. Xin G, Shi W, Xu LX, Su Y, Yan LJ, Li KS. Serum BAFF is elevated in patients with IgA nephropathy and associated with clinical and histopathological features. J Nephrol. 2013 Jul-Aug;26(4):683-90. eng. Epub 2012/10/09. doi:10.5301/jn.5000218. Cited in: Pubmed; PMID 23042433.
235. Vincent FB, Kandane-Rathnayake R, Hoi AY, Slavin L, Godsell JD, Kitching AR, Harris J, Nelson CL, Jenkins AJ, Chrysostomou A, Hibbs ML, Kerr PG, Rischmueller M, Mackay F, Morand EF. Urinary B-cell-activating factor of the tumour necrosis factor family (BAFF) in systemic lupus erythematosus. Lupus. 2018 Nov;27(13):2029-2040. eng. Epub 2018/10/12. doi:10.1177/0961203318804885. Cited in: Pubmed; PMID 30301439.

236. McCarthy DD, Chiu S, Gao Y, Summers-deLuca LE, Gommerman JL. BAFF induces a hyper-IgA syndrome in the intestinal lamina propria concomitant with IgA deposition in the kidney independent of LIGHT. Cellular Immunology. 2006 2006/06/01/;241(2):85-94. doi:https://doi.org/10.1016/j.cellimm.2006.08.002.
237. McCarthy DD, Kujawa J, Wilson C, Papandile A, Poreci U, Porfilio EA, Ward L, Lawson MA, Macpherson AJ, McCoy KD, Pei Y, Novak L, Lee JY, Julian BA, Novak J, Ranger A, Gommerman JL, Browning JL. Mice overexpressing BAFF develop a commensal flora-dependent, IgA-associated nephropathy. J Clin Invest. 2011 Oct;121(10):3991-4002. eng. Epub 2011/09/02. doi:10.1172/jci45563. Cited in: Pubmed; PMID 21881212.
238. Phatak S, Chaurasia S, Mishra SK, Gupta R, Agrawal V, Aggarwal A, Misra R. Urinary B cell activating factor (BAFF) and a proliferation-inducing ligand (APRIL): potential biomarkers of active lupus nephritis. Clin Exp Immunol. 2017 Mar;187(3):376-382. eng. Epub 2016/11/03. doi:10.1111/cei.12894. Cited in: Pubmed; PMID 27804111.
239. Huard B, Arlettaz L, Ambrose C, Kindler V, Mauri D, Roosnek E, Tschopp J, Schneider P, French LE. BAFF production by antigen-presenting cells provides T cell co-stimulation. Int Immunol. 2004 Mar;16(3):467-75. eng. Epub 2004/02/24. doi:10.1093/intimm/dxh043. Cited in: Pubmed; PMID 14978020.
240. Zheng N, Wang D, Ming H, Zhang H, Yu X. BAFF promotes proliferation of human mesangial cells through interaction with BAFF-R. BMC Nephrol. 2015 May 15;16:72. eng. Epub 2015/05/16. doi:10.1186/s12882-015-0064-y. Cited in: Pubmed; PMID 25975951.
241. Xu H, He X, Zhao W, Guo H, Shi Q, Zhu Y, Zhang X. CD256 can be found in antibody-mediated renal allograft rejection tissues. Clin Lab. 2012;58(5-6):411-8. eng. Epub 2012/07/13. Cited in: Pubmed; PMID 22783569.
242. Furie RA, Leon G, Thomas M, Petri MA, Chu AD, Hislop C, Martin RS, Scheinberg MA. A phase 2, randomised, placebo-controlled clinical trial of blisibimod, an inhibitor of B cell activating factor, in patients with moderate-to-severe systemic lupus erythematosus, the PEARL-SC study. Ann Rheum Dis. 2015 Sep;74(9):1667-75. eng. Epub 2014/04/22. doi:10.1136/annrheumdis-2013-205144. Cited in: Pubmed; PMID 24748629.
243. Merrill JT, Shanahan WR, Scheinberg M, Kalunian KC, Wofsy D, Martin RS. Phase III trial results with blisibimod, a selective inhibitor of B-cell activating factor, in subjects with systemic lupus erythematosus (SLE): results from a randomised, double-blind, placebo-controlled trial. Ann Rheum Dis. 2018 Jun;77(6):883-889. eng. Epub 2018/03/23. doi:10.1136/annrheumdis-2018-213032. Cited in: Pubmed; PMID 29563108.
244. Barratt J, Tumlin JA, Suzuki Y, Kao A, Aydemir A, Zima Y, Appel G. MO039THE 24-WEEK INTERIM ANALYSIS RESULTS OF A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED PHASE II STUDY OF ATACICEPT IN PATIENTS WITH IGA NEPHROPATHY AND PERSISTENT PROTEINURIA. Nephrology Dialysis Transplantation. 2020;35(Supplement_3):gfaa140.MO039. doi:10.1093/ndt/gfaa140.MO039.
245. Klauzinska M, Bertolette D, Tippireddy S, Strizzi L, Gray PC, Gonzales M, Duroux M, Ruvo M, Wechselberger C, Castro NP, Rangel MC, Focà A, Sandomenico A, Hendrix MJ, Salomon D, Cuttitta F. Cripto-1: an extracellular protein - connecting the sequestered biological dots. Connect Tissue Res. 2015;56(5):364-80. eng. Epub 2015/09/04. doi:10.3109/03008207.2015.1077239. Cited in: Pubmed; PMID 26327334.
246. Zhang D-m, Bao Y-L, Yu C-L, Wang Y-m, Song Z-B. Cripto-1 modulates macrophage cytokine secretion and phagocytic activity via NF-κB signaling. Immunologic Research. 2016 2016/02/01;64(1):104-114. doi:10.1007/s12026-015-8724-3.
247. Aydın B, Winters SJ. Sex Hormone-Binding Globulin in Children and Adolescents. J Clin Res Pediatr Endocrinol. 2016;8(1):1-12. eng. Epub 2015/01/18. doi:10.4274/jcrpe.2764. Cited in: Pubmed; PMID 26761949.
248. Yamazaki H, Kushiyama A, Sakoda H, Fujishiro M, Yamamotoya T, Nakatsu Y, Kikuchi T, Kaneko S, Tanaka H, Asano T. Protective Effect of Sex Hormone-Binding Globulin against Metabolic Syndrome: In Vitro Evidence Showing Anti-Inflammatory and Lipolytic Effects on Adipocytes and Macrophages. Mediators Inflamm. 2018;2018:3062319. eng. Epub 2018/07/27. doi:10.1155/2018/3062319. Cited in: Pubmed; PMID 30046278.
249. Simó R, Barbosa-Desongles A, Sáez-Lopez C, Lecube A, Hernandez C, Selva DM. Molecular Mechanism of TNFα-Induced Down-Regulation of SHBG Expression. Molecular Endocrinology. 2012;26(3):438-446. doi:10.1210/me.2011-1321.
250. Simó R, Barbosa-Desongles A, Hernandez C, Selva DM. IL1β down-regulation of sex hormone-binding globulin production by decreasing HNF-4α via MEK-1/2 and JNK MAPK pathways. Molecular Endocrinology. 2012;26(11):1917-1927.
251. Mirone L, Altomonte L, D'Agostino P, Zoli A, Barini A, Magaro M. A study of serum androgen and cortisol levels in female patients with rheumatoid arthritis. Correlation with disease activity [Article]. Clinical Rheumatology. 1996;15(1):15-19. doi:10.1007/BF02231678.
252. Carlström K, Hedin PJ, Jönsson L, Lerndal T, Lien J, Weitoft T, Axelson M. Endocrine effects of the podophyllotoxine derivative drug CPH 82 (Reumacon®) in patients with rheumatoid arthritis [Article]. Scandinavian Journal of Rheumatology. 2000;29(2):89-94. doi:10.1080/030097400750001888.
253. Sattar N, Crompton P, Cherry L, Kane D, Lowe G, McInnes IB. Effects of tumor necrosis factor blockade on cardiovascular risk factors in psoriatic arthritis: A double-blind, placebo-controlled study [https://doi.org/10.1002/art.22447]. Arthritis & Rheumatism. 2007 2007/03/01;56(3):831-839. doi:https://doi.org/10.1002/art.22447.
254. Pugeat M, Nader N, Hogeveen K, Raverot G, Déchaud H, Grenot C. Sex hormone-binding globulin gene expression in the liver: Drugs and the metabolic syndrome. Molecular and Cellular Endocrinology. 2010 2010/03/05/;316(1):53-59. doi:https://doi.org/10.1016/j.mce.2009.09.020.
255. Urushihara M, Kagami S. Role of the intrarenal renin–angiotensin system in the progression of renal disease. Pediatric Nephrology. 2017 2017/09/01;32(9):1471-1479. doi:10.1007/s00467-016-3449-7.
256. Inker LA, Astor BC, Fox CH, Isakova T, Lash JP, Peralta CA, Kurella Tamura M, Feldman HI. KDOQI US Commentary on the 2012 KDIGO Clinical Practice Guideline for the Evaluation and Management of CKD. American Journal of Kidney Diseases. 2014 2014/05/01/;63(5):713-735. doi:https://doi.org/10.1053/j.ajkd.2014.01.416.
257. Békássy ZD, Kristoffersson A-C, Rebetz J, Tati R, Olin AI, Karpman D. Aliskiren inhibits renin-mediated complement activation. Kidney International. 2018 2018/10/01/;94(4):689-700. doi:https://doi.org/10.1016/j.kint.2018.04.004.
258. Lee YH, Choi SJ, Ji JD, Song GG. Associations between the angiotensin-converting enzyme insertion/deletion polymorphism and susceptibility to vasculitis: a meta-analysis. Journal of the Renin-Angiotensin-Aldosterone System. 2012 2012/03/01;13(1):196-201. doi:10.1177/1470320311434240.
259. Lantelme P, Rohrwasser A, Gociman B, Hillas E, Cheng T, Petty G, Thomas J, Xiao S, Ishigami T, Herrmann T, Terreros DA, Ward K, Lalouel JM. Effects of dietary sodium and genetic background on angiotensinogen and Renin in mouse. Hypertension. 2002 May;39(5):1007-14. eng. Epub 2002/05/23. doi:10.1161/01.hyp.0000016177.20565.a0. Cited in: Pubmed; PMID 12019284.
260. Nishiyama A, Konishi Y, Ohashi N, Morikawa T, Urushihara M, Maeda I, Hamada M, Kishida M, Hitomi H, Shirahashi N, Kobori H, Imanishi M. Urinary angiotensinogen reflects the activity of intrarenal renin-angiotensin system in patients with IgA nephropathy. Nephrol Dial Transplant. 2011 Jan;26(1):170-7. eng. Epub 2010/07/10. doi:10.1093/ndt/gfq371. Cited in: Pubmed; PMID 20615910.
261. Yi-fei M, Yu-feng L, Gui-mei G, Ya-ju Z, Ying-liang G, Yu D. Changes of new urinary biomarkers in children with Henoch-Schonlein purpura nephritis. JOURNAL OF SHANGHAI JIAOTONG UNIVERSITY (MEDICAL SCIENCE). 2020;40(06):841.

262. Mao YN, Liu WEI, Li YG, Jia GC, Zhang Z, Guan YJ, Zhou XF, Liu YF. Urinary angiotensinogen levels in relation to renal involvement of Henoch-Schonlein purpura in children [https://doi.org/10.1111/j.1440-1797.2011.01515.x]. Nephrology. 2012 2012/01/01;17(1):53-57. doi:https://doi.org/10.1111/j.1440-1797.2011.01515.x.
263. van der Vorst EPC. High-Density Lipoproteins and Apolipoprotein A1. In: Hoeger U, Harris JR, editors. Vertebrate and Invertebrate Respiratory Proteins, Lipoproteins and other Body Fluid Proteins. Cham: Springer International Publishing; 2020. p. 399-420.
264. Kon V, Yang H-C, Smith LE, Vickers KC, Linton MF. High-Density Lipoproteins in Kidney Disease. International journal of molecular sciences. 2021;22(15):8201. eng. doi:10.3390/ijms22158201. Cited in: Pubmed; PMID 34360965.
265. Georgila K, Vyrla D, Drakos E. Apolipoprotein A-I (ApoA-I), Immunity, Inflammation and Cancer. Cancers (Basel). 2019;11(8):1097. eng. doi:10.3390/cancers11081097. Cited in: Pubmed; PMID 31374929.
266. Clark AJ, Jabs K, Hunley TE, Jones DP, VanDeVoorde RG, Anderson C, Du L, Zhong J, Fogo AB, Yang H, Kon V. Urinary apolipoprotein AI in children with kidney disease. Pediatr Nephrol. 2019;34(11):2351-2360. eng. Epub 2019/06/23. doi:10.1007/s00467-019-04289-5. Cited in: Pubmed; PMID 31230128.
267. Lopez-Hellin J, Cantarell C, Jimeno L, Sanchez-Fructuoso A, Puig-Gay N, Guirado L, Vilariño N, Gonzalez-Roncero FM, Mazuecos A, Lauzurica R, Burgos D, Plumed JS, Jacobs-Cacha C, Jimenez C, Fernandez A, Fernandez-Alvarez P, Torregrosa V, Nieto JL, Meseguer A, Alonso A. A form of apolipoprotein a-I is found specifically in relapses of focal segmental glomerulosclerosis following transplantation. Am J Transplant. 2013 Feb;13(2):493-500. eng. Epub 2012/12/05. doi:10.1111/j.1600-6143.2012.04338.x. Cited in: Pubmed; PMID 23205849.
268. Zhu W, Liu M, Wang GC, Peng B, Yan Y, Che JP, Ma QW, Yao XD, Zheng JH. Fibrinogen alpha chain precursor and apolipoprotein A-I in urine as biomarkers for noninvasive diagnosis of calcium oxalate nephrolithiasis: a proteomics study. Biomed Res Int. 2014;2014:415651. eng. Epub 2014/08/26. doi:10.1155/2014/415651. Cited in: Pubmed; PMID 25147800.
269. Nielsen R, Christensen EI, Birn H. Megalin and cubilin in proximal tubule protein reabsorption: from experimental models to human disease. Kidney Int. 2016 Jan;89(1):58-67. eng. Epub 2016/01/14. doi:10.1016/j.kint.2015.11.007. Cited in: Pubmed; PMID 26759048.
270. Puig-Gay N, Jacobs-Cacha C, Sellarès J, Guirado L, González Roncero F, Jiménez C, Zárraga S, Paul J, Lauzurica R, Alonso Á, Fernández A, Beneyto I, Mazuecos A, Hernández D, Rodriguez-Benot A, Franco A, Jimeno L, Crespo M, Meseguer A, Moreso F, Seron D, Lopez-Hellin J, Cantarell C. Apolipoprotein A-Ib as a biomarker of focal segmental glomerulosclerosis recurrence after kidney transplantation: diagnostic performance and assessment of its prognostic value - a multi-centre cohort study. Transpl Int. 2019 Mar;32(3):313-322. eng. Epub 2018/11/10. doi:10.1111/tri.13372. Cited in: Pubmed; PMID 30411406.
271. Davin J-C, Ten Berge IJ, Weening JJ. What is the difference between IgA nephropathy and Henoch-Schönlein purpura nephritis? Kidney International. 2001 2001/03/01/;59(3):823-834. doi:https://doi.org/10.1046/j.1523-1755.2001.059003823.x.
272. Guo RF, Ward PA. Role of C5a in inflammatory responses. Annu Rev Immunol. 2005;23:821-52. eng. Epub 2005/03/18. doi:10.1146/annurev.immunol.23.021704.115835. Cited in: Pubmed; PMID 15771587.
273. Medjeral-Thomas NR, Cook HT, Pickering MC. Complement activation in IgA nephropathy. Semin Immunopathol. 2021;43(5):679-690. eng. Epub 2021/08/11. doi:10.1007/s00281-021-00882-9. Cited in: Pubmed; PMID 34379175.
274. Zhu L, Zhai YL, Wang FM, Hou P, Lv JC, Xu DM, Shi SF, Liu LJ, Yu F, Zhao MH, Novak J, Gharavi AG, Zhang H. Variants in Complement Factor H and Complement Factor H-Related Protein Genes, CFHR3 and CFHR1, Affect Complement Activation in IgA Nephropathy. J Am Soc Nephrol. 2015 May;26(5):1195-204. eng. Epub 2014/09/11. doi:10.1681/asn.2014010096. Cited in: Pubmed; PMID 25205734.
275. Kiryluk K, Li Y, Sanna-Cherchi S, Rohanizadegan M, Suzuki H, Eitner F, Snyder HJ, Choi M, Hou P, Scolari F, Izzi C, Gigante M, Gesualdo L, Savoldi S, Amoroso A, Cusi D, Zamboli P, Julian BA, Novak J, Wyatt RJ, Mucha K, Perola M, Kristiansson K, Viktorin A, Magnusson PK, Thorleifsson G, Thorsteinsdottir U, Stefansson K, Boland A, Metzger M, Thibaudin L, Wanner C, Jager KJ, Goto S, Maixnerova D, Karnib HH, Nagy J, Panzer U, Xie J, Chen N, Tesar V, Narita I, Berthoux F, Floege J, Stengel B, Zhang H, Lifton RP, Gharavi AG. Geographic differences in genetic susceptibility to IgA nephropathy: GWAS replication study and geospatial risk analysis. PLoS Genet. 2012;8(6):e1002765. eng. Epub 2012/06/28. doi:10.1371/journal.pgen.1002765. Cited in: Pubmed; PMID 22737082.
276. Tortajada A, Gutiérrez E, Goicoechea de Jorge E, Anter J, Segarra A, Espinosa M, Blasco M, Roman E, Marco H, Quintana LF, Gutiérrez J, Pinto S, Lopez-Trascasa M, Praga M, Rodriguez de Córdoba S. Elevated factor H-related protein 1 and factor H pathogenic variants decrease complement regulation in IgA nephropathy. Kidney Int. 2017 Oct;92(4):953-963. eng. Epub 2017/06/24. doi:10.1016/j.kint.2017.03.041. Cited in: Pubmed; PMID 28637589.
277. Gharavi AG, Kiryluk K, Choi M, Li Y, Hou P, Xie J, Sanna-Cherchi S, Men CJ, Julian BA, Wyatt RJ, Novak J, He JC, Wang H, Lv J, Zhu L, Wang W, Wang Z, Yasuno K, Gunel M, Mane S, Umlauf S, Tikhonova I, Beerman I, Savoldi S, Magistroni R, Ghiggeri GM, Bodria M, Lugani F, Ravani P, Ponticelli C, Allegri L, Boscutti G, Frasca G, Amore A, Peruzzi L, Coppo R, Izzi C, Viola BF, Prati E, Salvadori M, Mignani R, Gesualdo L, Bertinetto F, Mesiano P, Amoroso A, Scolari F, Chen N, Zhang H, Lifton RP. Genome-wide association study identifies susceptibility loci for IgA nephropathy. Nat Genet. 2011 Mar 13;43(4):321-7. eng. Epub 2011/03/15. doi:10.1038/ng.787. Cited in: Pubmed; PMID 21399633.
278. Rayego-Mateos S, Rodrigues-Diez R, Morgado-Pascual JL, Valentijn F, Valdivielso JM, Goldschmeding R, Ruiz-Ortega M. Role of Epidermal Growth Factor Receptor (EGFR) and Its Ligands in Kidney Inflammation and Damage. Mediators Inflamm. 2018;2018:8739473. eng. Epub 2019/01/24. doi:10.1155/2018/8739473. Cited in: Pubmed; PMID 30670929.
279. Harskamp LR, Gansevoort RT, van Goor H, Meijer E. The epidermal growth factor receptor pathway in chronic kidney diseases. Nature Reviews Nephrology. 2016 2016/08/01;12(8):496-506. doi:10.1038/nrneph.2016.91.
280. Bui TM, Wiesolek HL, Sumagin R. ICAM-1: A master regulator of cellular responses in inflammation, injury resolution, and tumorigenesis. J Leukoc Biol. 2020 Sep;108(3):787-799. eng. Epub 2020/03/18. doi:10.1002/jlb.2mr0220-549r. Cited in: Pubmed; PMID 32182390.
281. Xu Y, Li S. Blockade of ICAM-1: A novel way of vasculitis treatment. Biochemical and Biophysical Research Communications. 2009 2009/04/17/;381(4):459-461. doi:https://doi.org/10.1016/j.bbrc.2009.02.111.
282. Gok F, Ugur Y, Ozen S, Dagdeviren A. Pathogenesis-related adhesion molecules in Henoch–Schonlein vasculitis. Rheumatology International. 2008 2008/02/01;28(4):313-316. doi:10.1007/s00296-007-0437-z.
283. Gattorno M, Vignola S, Barbano G, Sormani MP, Sabatini F, Buoncompagni A, Picco P, Pistoia V. Tumor necrosis factor induced adhesion molecule serum concentrations in Henoch-Schönlein purpura and pediatric systemic lupus erythematosus. J Rheumatol. 2000 Sep;27(9):2251-5. eng. Epub 2000/09/16. Cited in: Pubmed; PMID 10990243.
284. Hughes CE, Nibbs RJB. A guide to chemokines and their receptors. FEBS J. 2018;285(16):2944-2971. eng. Epub 2018/04/24. doi:10.1111/febs.14466. Cited in: Pubmed; PMID 29637711.
285. Norlander AE, Saleh MA, Madhur MS. CXCL16: a chemokine-causing chronic kidney disease. Hypertension (Dallas, Tex : 1979). 2013;62(6):1008-1010. eng. Epub 2013/09/23. doi:10.1161/HYPERTENSIONAHA.113.01954. Cited in: Pubmed; PMID 24060889.
286. Xia Y, Entman ML, Wang Y. Critical role of CXCL16 in hypertensive kidney injury and fibrosis. Hypertension. 2013 Dec;62(6):1129-37. eng. Epub 2013/09/26. doi:10.1161/hypertensionaha.113.01837. Cited in: Pubmed; PMID 24060897.

287. Luo R, Yang Y, Cheng Y-C, Chang D, Liu T-T, Li Y-Q, Dai W, Zuo M-Y, Xu Y-L, Zhang C-X, Ge S-W, Xu G. Plasma chemokine CXC motif-ligand 16 as a predictor of renal prognosis in immunoglobulin A nephropathy. Ann Transl Med. 2020;8(6):381-381. eng. doi:10.21037/atm.2020.02.05. Cited in: Pubmed; PMID 32355825.
288. Zhu L, Zhang Q, Shi S, Liu L, Lv J, Zhang H. Synergistic effect of mesangial cell-induced CXCL1 and TGF-β1 in promoting podocyte loss in IgA nephropathy. PloS one. 2013;8(8):e73425-e73425. eng. doi:10.1371/journal.pone.0073425. Cited in: Pubmed; PMID 24023680.
289. Zhao Y, Zhu L, Zhou T, Zhang Q, Shi S, Liu L, Lv J, Zhang H. Urinary CXCL1: a novel predictor of IgA nephropathy progression. PLoS One. 2015;10(3):e0119033. eng. Epub 2015/03/31. doi:10.1371/journal.pone.0119033. Cited in: Pubmed; PMID 25816025.
290. Yüksel B, Özbek MN, Mungan NÖ, Darendeliler F, Budan B, Bideci A, Çetinkaya E, Berberoğlu M, Evliyaoğlu O, Yeşilkaya E, Arslanoğlu İ, Darcan Ş, Bundak R, Ercan O. Serum IGF-1 and IGFBP-3 levels in healthy children between 0 and 6 years of age. J Clin Res Pediatr Endocrinol. 2011;3(2):84-88. eng. Epub 2011/06/08. doi:10.4274/jcrpe.v3i2.17. Cited in: Pubmed; PMID 21750637.
291. Wang S, Chi K, Wu D, Hong Q. Insulin-Like Growth Factor Binding Proteins in Kidney Disease [Review]. Frontiers in Pharmacology. 2021 2021-December-22;12. English. doi:10.3389/fphar.2021.807119.
292. Zhang Z, Wang H, Zhang L, Crew R, Zhang N, Liu X, Jiang Y. Serum Levels of Soluble ST2 and IL-10 Are Associated with Disease Severity in Patients with IgA Nephropathy. J Immunol Res. 2016;2016:6540937-6540937. eng. Epub 2016/11/09. doi:10.1155/2016/6540937. Cited in: Pubmed; PMID 27900334.
293. Chen T, Jia RZ, Guo ZP, Cao N, Li MM, Jiao XY. Elevated serum interleukin-33 levels in patients with Henoch-Schönlein purpura. Arch Dermatol Res. 2013 Mar;305(2):173-7. eng. Epub 2012/07/28. doi:10.1007/s00403-012-1268-7. Cited in: Pubmed; PMID 22836779.
294. Zhang S, Breidenbach JD, Russell BH, George J, Haller ST. CD40/CD40L Signaling as a Promising Therapeutic Target for the Treatment of Renal Disease. J Clin Med. 2020;9(11):3653. eng. doi:10.3390/jcm9113653. Cited in: Pubmed; PMID 33202988.
295. Yang B, Tan X, Xiong X, Wu D, Zhang G, Wang M, Dong S, Liu W, Yang H, Li Q. Effect of CD40/CD40L signaling on IL-10-producing regulatory B cells in Chinese children with Henoch-Schönlein purpura nephritis. Immunologic Research. 2017 2017/06/01;65(3):592-604. doi:10.1007/s12026-016-8877-8.
296. Szmitko PE, Wang CH, Weisel RD, de Almeida JR, Anderson TJ, Verma S. New markers of inflammation and endothelial cell activation: Part I. Circulation. 2003 Oct 21;108(16):1917-23. eng. Epub 2003/10/22. doi:10.1161/01.Cir.0000089190.95415.9f. Cited in: Pubmed; PMID 14568885.
297. Eddy AA, Fogo AB. Plasminogen Activator Inhibitor-1 in Chronic Kidney Disease: Evidence and Mechanisms of Action. Journal of the American Society of Nephrology. 2006;17(11):2999. doi:10.1681/ASN.2006050503.
298. Peters H, Border WA, Noble NA. Angiotensin II blockade and low-protein diet produce additive therapeutic effects in experimental glomerulonephritis. Kidney Int. 2000 Apr;57(4):1493-501. eng. Epub 2000/04/12. doi:10.1046/j.1523-1755.2000.00994.x. Cited in: Pubmed; PMID 10760085.
299. Ihm C-G, Jeong KW, Lee SH, Lee TW, Park JK. Effects of therapeutic agents on the inflammatory and fibrogenic factors in IgA nephropathy [https://doi.org/10.1111/j.1440-1797.2007.00878.x]. Nephrology. 2007 2007/12/01;12(s3):S25-S26. doi:https://doi.org/10.1111/j.1440-1797.2007.00878.x.
300. Yoshino J, Monkawa T, Tsuji M, Hayashi M, Saruta T. Leukemia Inhibitory Factor Is Involved in Tubular Regeneration after Experimental Acute Renal Failure. Journal of the American Society of Nephrology. 2003;14(12):3090. doi:10.1097/01.ASN.0000101180.96787.02.
301. Yamada K, Huang ZQ, Raska M, Reily C, Anderson JC, Suzuki H, Kiryluk K, Gharavi AG, Julian BA, Willey CD, Novak J. Leukemia Inhibitory Factor Signaling Enhances Production of Galactose-Deficient IgA1 in IgA Nephropathy. Kidney Dis (Basel). 2020;6(3):168-180. eng. Epub 2020/04/16. doi:10.1159/000505748. Cited in: Pubmed; PMID 32523959.
302. Harriman GR, Kunimoto DY, Elliott JF, Paetkau V, Strober W. The role of IL-5 in IgA B cell differentiation. J Immunol. 1988 May 1;140(9):3033-9. eng. Epub 1988/05/01. Cited in: Pubmed; PMID 3258891.
303. Wang J, Li Y, Chen Y, Dai X, Di Y, Shen M, Ying Q, Fu S, Li Y. Urinary Macrophage Migration Inhibitory Factor as a Noninvasive Biomarker in Pediatric Henoch-Schönlein Purpura Nephritis. J Clin Rheumatol. 2017 Aug;23(5):258-261. eng. Epub 2017/07/13. doi:10.1097/rhu.0000000000000570. Cited in: Pubmed; PMID 28700526.
304. Qin Y-H, Zhou T-B, Lei F-Y, Huang W-F, Zhao Y-J, Lin F-Q, Su L-N. Cut-off values for serum matrix metalloproteinase-9: Is there a threshold to predict renal involvement for Henoch–Schonlein purpura in children? [https://doi.org/10.1111/j.1440-1797.2010.01360.x]. Nephrology. 2011 2011/01/01;16(1):93-99. doi:https://doi.org/10.1111/j.1440-1797.2010.01360.x.
305. Dyga K, Machura E, Świętochowska E, Szczepańska M. Analysis of the association between kidney injury biomarkers concentration and nephritis in immunoglobulin A vasculitis: A pediatric cohort study. Int J Rheum Dis. 2020 Aug;23(9):1184-1193. eng. Epub 2020/07/07. doi:10.1111/1756-185x.13904. Cited in: Pubmed; PMID 32627422.
306. García-Porrúa C, Calviño MC, Llorca J, Couselo JM, González-Gay MA. Henoch-Schönlein purpura in children and adults: Clinical differences in a defined population. Seminars in Arthritis and Rheumatism. 2002 2002/12/01/;32(3):149-156. doi:https://doi.org/10.1053/sarh.2002.33980.
307. Gonzalez-Gay MA, Calviño MC, Vazquez-Lopez ME, Garcia-Porrua C, Fernandez-Iglesias JL, Dierssen T, Llorca J. Implications of upper respiratory tract infections and drugs in the clinical spectrum of Henoch-Schönlein purpura in children. Clin Exp Rheumatol. 2004 Nov-Dec;22(6):781-4. eng. Epub 2005/01/11. Cited in: Pubmed; PMID 15638057.
308. Cakir M, Orhan F, Mungan I, Sonmez FM, Aslan Y, Kalyoncu M, Erduran E, Gedik Y, Okten A. Henoch-Schönlein purpura in north-eastern Turkey. Ann Trop Paediatr. 2006 Mar;26(1):59-65. eng. Epub 2006/02/24. doi:10.1179/146532806x90628. Cited in: Pubmed; PMID 16494706.
309. Jauhola O, Ronkainen J, Koskimies O, Ala-Houhala M, Arikoski P, Hölttä T, Jahnukainen T, Rajantie J, Ormälä T, Turtinen J, Nuutinen M. Renal manifestations of Henoch-Schonlein purpura in a 6-month prospective study of 223 children. Arch Dis Child. 2010 Nov;95(11):877-82. eng. Epub 2010/09/21. doi:10.1136/adc.2009.182394. Cited in: Pubmed; PMID 20852275.
310. Shin JI, Lee SJ, Lee JS, Kim KH. Intravenous dexamethasone followed by oral prednisolone versus oral prednisolone in the treatment of childhood Henoch-Schönlein purpura. Rheumatol Int. 2011 Nov;31(11):1429-32. eng. Epub 2010/05/14. doi:10.1007/s00296-010-1507-1. Cited in: Pubmed; PMID 20464400.
311. Lardhi AA. Henoch-Schonlein purpura in children from the eastern province of Saudi Arabia. Saudi Med J. 2012 Sep;33(9):973-8. eng. Epub 2012/09/12. Cited in: Pubmed; PMID 22964809.
312. Yılmaz A, Aytac M, Ekinci Z. Retrospective Assessment of Children with Henoch-Schonlein Purpura in and around Kocaeli Province and Comparison with Literature. Erciyes Tıp Dergisi/Erciyes Medical Journal. 2014 07/22;36:62-67. doi:10.5152/etd.2013.63.
313. Lee YH, Kim YB, Koo JW, Chung JY. Henoch-Schonlein Purpura in Children Hospitalized at a Tertiary Hospital during 2004-2015 in Korea: Epidemiology and Clinical Management. Pediatr Gastroenterol Hepatol Nutr. 2016 Sep;19(3):175-185. eng. Epub 2016/10/16. doi:10.5223/pghn.2016.19.3.175. Cited in: Pubmed; PMID 27738599.

314. Ma DQ, Li Y, Han ZG, Zheng M, Xu N, Fan XJ. Analysis on kidney injury-related clinical risk factors and evaluation on the therapeutic effects of hemoperfusion in children with Henoch-Schonlein purpura. Eur Rev Med Pharmacol Sci. 2017 Oct;21(17):3894-3899. eng. Epub 2017/10/05. Cited in: Pubmed; PMID 28975974.
315. Xu Y, Wang JJ, Liu FF, Wu Y, Wu YF, Samadli S, Luo HH, Zhang DD, Hu P. Predisposing factors of childhood Henoch-Schönlein purpura in Anhui province, China. J Investig Med. 2019 Apr;67(4):771-778. eng. Epub 2018/12/12. doi:10.1136/jim-2018-000906. Cited in: Pubmed; PMID 30530785.
316. Rhim JW, Lee YT, Kang HM, Suh JS, Lee KY. Changes in clinical features in Henoch-Schönlein purpura during three decades: an observational study at a single hospital in Korea. Clin Rheumatol. 2019 Oct;38(10):2811-2818. eng. Epub 2019/06/19. doi:10.1007/s10067-019-04628-9. Cited in: Pubmed; PMID 31209707.
317. Ekinci RMK, Balci S, Sari Gokay S, Yilmaz HL, Dogruel D, Altintas DU, Yilmaz M. Do practical laboratory indices predict the outcomes of children with Henoch-Schönlein purpura? Postgrad Med. 2019 May;131(4):295-298. eng. Epub 2019/04/26. doi:10.1080/00325481.2019.1609814. Cited in: Pubmed; PMID 31021301.
318. Karadağ Ş G, Tanatar A, Sönmez HE, Çakmak F, Kıyak A, Yavuz S, Çakan M, Ayaz NA. The clinical spectrum of Henoch-Schönlein purpura in children: a single-center study. Clin Rheumatol. 2019 Jun;38(6):1707-1714. eng. Epub 2019/02/09. doi:10.1007/s10067-019-04460-1. Cited in: Pubmed; PMID 30734116.
319. Wang JJ, Xu Y, Liu FF, Wu Y, Samadli S, Wu YF, Luo HH, Zhang DD, Hu P. Association of the infectious triggers with childhood Henoch-Schonlein purpura in Anhui province, China. J Infect Public Health. 2020 Jan;13(1):110-117. eng. Epub 2019/07/25. doi:10.1016/j.jiph.2019.07.004. Cited in: Pubmed; PMID 31337540.
320. Zhang Y, Xia G, Nie X, Zeng Y, Chen Y, Qian Y, Chen G, Huang J, Wang C, Zhang C, Huang X, Yang Y, Qiu X, Yang F, Chen J, Hu J. Differences in Manifestations and Gut Microbiota Composition Between Patients With Different Henoch-Schonlein Purpura Phenotypes. Front Cell Infect Microbiol. 2021;11:641997. eng. Epub 2021/07/20. doi:10.3389/fcimb.2021.641997. Cited in: Pubmed; PMID 34277463.
321. Sestan M, Kifer N, Frkovic M, Sapina M, Srsen S, Batnozic Varga M, Ovuka A, Held M, Gudelj Gracanin A, Kozmar A, Bulimbasic S, Coric M, Laskarin G, Gagro A, Jelusic M. Gastrointestinal involvement and its association with the risk for nephritis in IgA vasculitis. Ther Adv Musculoskelet Dis. 2021;13:1759720x211024828. eng. Epub 2021/07/16. doi:10.1177/1759720x211024828. Cited in: Pubmed; PMID 34262620.

8. Appendices

8.1. **Appendix 1**

The published version of chapter 3: Urinary Protein Array Analysis to Identify Key Inflammatory Markers in Children with IgA Vasculitis Nephritis.

children

MDPI

Article

Urinary Protein Array Analysis to Identify Key Inflammatory Markers in Children with IgA Vasculitis Nephritis

Julien Marro [1], Andrew J. Chetwynd [1], Rachael D. Wright [1], Silothabo Dliso [2] and Louise Oni [1,3,*]

[1] Department of Women's and Children's Health, Institute of Life Course and Medical Sciences, University of Liverpool, Liverpool L12 2AP, UK; j.g.marro@liverpool.ac.uk (J.M.); a.j.chetwynd@liverpool.ac.uk (A.J.C.); rachael.wright@liverpool.ac.uk (R.D.W.)
[2] NIHR Alder Hey Clinical Research Facility, Clinical Research Division, Alder Hey Children's NHS Foundation Trust, Liverpool L14 5AB, UK; silothabo.dliso@alderhey.nhs.uk
[3] Department of Paediatric Nephrology, Alder Hey Children's NHS Foundation Trust Hospital, Liverpool L14 5AB, UK
* Correspondence: louise.oni@liverpool.ac.uk; Tel.: +44-(0)151-252-5441

Abstract: Chronic kidney disease is a recognised complication of immunoglobulin A vasculitis, (IgAV; formerly Henoch–Schonlein purpura—HSP). The pathophysiology of IgAV and why some patients develop significant renal involvement remains largely unknown. Identifying urinary inflammatory markers could direct targets for earlier intervention. The aim of this cross-sectional exploratory study was to perform a large protein array analysis to identify urinary markers to provide insight into the mechanisms of kidney inflammation in children with established IgAV nephritis (IgAVN). Determination of the relative levels of 124 key proteins was performed using commercially available proteome profiler array kits. Twelve children were recruited: IgAVN, $n = 4$; IgAV without nephritis (IgAVwoN), $n = 4$; healthy controls (HCs), $n = 4$. The urinary concentrations of twenty proteins were significantly different in IgAVN compared to IgAVwoN. The largest fold changes were reported for B-cell activating factor (BAFF), Cripto-1, sex-hormone-binding globulin and angiotensinogen. The urinary levels of complement components C5/C5a and factor D were also significantly elevated in patients with IgAVN. A total of 69 urinary proteins significantly raised levels in comparisons made between IgAVN vs. HCs and nine proteins in IgAVwoN vs. HCs, respectively. This study identified key urinary proteins potentially involved in IgAVN providing new insight into the pathophysiology. Further longitudinal studies with larger cohorts are needed to quantitatively analyse these biomarkers.

Keywords: IgA vasculitis; Henoch–Schonlein purpura; IgAV; HSP; nephritis; children; urine; biomarker

check for updates

Citation: Marro, J.; Chetwynd, A.J.; Wright, R.D.; Dliso, S.; Oni, L. Urinary Protein Array Analysis to Identify Key Inflammatory Markers in Children with IgA Vasculitis Nephritis. *Children* **2022**, *9*, 622. https://doi.org/10.3390/children9050622

Academic Editors: Licia Peruzzi and Yohei Ikezumi

Received: 1 March 2022
Accepted: 25 April 2022
Published: 27 April 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

Immunoglobulin A vasculitis (IgAV; formerly Henoch–Schonlein purpura—HSP [1]) is an immune-mediated vasculitis affecting small blood vessels. It is the most common form of childhood vasculitis with an estimated annual incidence of 20.4 per 100,000 children and a peak age of onset at approximately 6 years old [2]. It usually presents with a purpuric non-blanching rash with lower limb predominance, and it is commonly associated with abdominal pain, arthralgia/arthritis and renal involvement [3]. The disease typically self-resolves over a period of weeks to months in over 90% of children [4]; hence, treatment is mainly supportive [5]. Renal involvement (i.e., IgAV nephritis and IgAVN) is the most serious long-term manifestation of IgAV. IgAVN occurs in 40 to 50% of children, and it can present with a spectrum of manifestations that include microscopic haematuria and/or proteinuria, nephritic/nephrotic syndrome [4,6] and, rarely, rapidly progressive glomerulonephritis [7]. Although renal involvement is mainly self-limiting, 1–2% of children progress to chronic kidney disease (CKD) stage 5 [8]. For this reason, common clinical practice is that all children with IgAV should have at least 6 months of urinalysis testing

and blood pressure monitoring following the first presentation [9,10]. The rising presence of proteinuria is suggestive of evolving nephritis, and the gold standard for confirming and grading the diagnosis is to perform a renal biopsy [5].

Histologically, IgAVN is characterised by mesangial deposition of pathogenic galactose-deficient IgA1 containing immune complexes, complement activation and neutrophil infiltrates in the glomerulus [11]. Yet, the exact pathophysiology of IgAV and the reasons why some patients develop significant renal involvement remains largely unknown. Identifying the variations in urinary proteome profiles between patients with and without nephritis may aid understanding of the pathophysiology of IgAVN and direct the identification of new treatment targets for future validation studies.

Urine offers an ideal biofluid to assess the presence of renal disease and to uncover potential biomarkers that may be more indicative of IgAVN and help elucidate mechanisms of disease. A recent systematic review of the existing literature highlighted that kidney injury molecule-1 (KIM-1), monocyte chemotactic protein-1 (MCP-1), N-acetyl-β-glucosaminidase (NAG) and urinary angiotensinogen (UAGT) seemed to be the most promising biomarkers in identifying the presence and/or severity of IgAVN [12]. The aim of this study was to perform a large exploratory protein array analysis of urinary markers of kidney inflammation and/or injury to provide insight into the pathophysiology of IgAV using a select cohort of children with established IgAVN.

2. Materials and Methods

2.1. Definitions and Patient Selection

Children were recruited as part of the IgA Vasculitis Study, a single-centre observational longitudinal study at Alder Hey Children's Hospital, Liverpool, UK, between 28 August 2019 and 13 October 2021. For this study, clinical data (i.e., history, medication, blood pressure, urinalysis, blood tests, histology reports, imaging report and disease progression) were collected at presentation and during follow up together with biosamples including urine.

Children of any sex aged <18 years old at diagnosis were eligible to take part in this study. Patients had to be diagnosed clinically with IgA vasculitis according to the EULAR/PRINTO/PRES criteria [3]. A minimum follow-up period of 12 months from sample collection was required, and a urine sample had to be obtained during this follow-up period. Patients were considered to have completed the 12 months follow up if they were discharged from the 6 month nurse-led HSP pathway [10] and did not have any further IgAV-related presentations to the institution. Exclusion criteria were as follows: (1) diagnosis of IgAV uncertain or in doubt; (2) other concurrent inflammatory or renal condition; (3) incomplete follow up; (4) urine sample obtained at a point greater than 12 months from diagnosis; (5) undergoing dialysis.

The patients with IgAV were subdivided according to the presence of nephritis. Nephritis (termed the "IgAVN" group) was defined as a urinary albumin to creatinine (UACR) > 30 mg/mmol at the time of sampling [3] and a kidney biopsy demonstrating IgAV prior to or within 7 days of sample collection. In order to identify cases with more severe renal inflammation, patients were selected from those with the highest UACR from the IgA Vasculitis study cohort. Patients with no renal involvement (termed the "IgAVwoN" group) were patients with a negative urine dipstick for protein and blood at the time of sampling and with an uncomplicated disease course, defined as no further presentation due to the fact of IgAV, a UACR < 10 mg/mmol at any point during their follow up and those who received no treatment apart from simple analgesia. A UACR of 0 mg/mmol was assumed for urine dipsticks negative for protein. Hypertension was defined as a systolic blood pressure above the 95th centile for the child's age, sex and height for <16 years old or >140 mmHg for children 16 years and older [13].

Healthy controls were recruited to provide a one-off, age and sex-matched clean-catch, midstream spot urine sample. These were children aged <18 years old who were attending

for noninflammatory day case investigations or surgery and with not known relevant medical history or not taking any regular medication.

2.2. Sample Processing

Healthy control urine samples were tested for bacterial contamination using urine dipstick testing, and they were discarded if they demonstrated positivity for leukocytes, nitrites, blood or >+1 for protein. Urine samples were centrifuged at 300× *g* for 10 min, transferred into a new falcon tube, centrifuged again at 300× *g* for 10 min and aliquoted into 1 mL sterile Eppendorf tubes for storage. Samples were stored at −80 °C and were thawed at room temperature and centrifuged for 5 min at 300× *g* on the day of the experiment.

2.3. Membrane Antibody Arrays

Human Kidney Biomarker Array Kit and Human XL Cytokine Array Kit (R&D Systems, Minneapolis, MN, USA) were used to assess the relative concentrations of 38 predefined markers of kidney damage and 105 inflammatory cytokines (total of 124 proteins, 19 were duplicated as they were evaluated in both kits). The full list of the proteins assessed can be found in supplementary Table S1. Both kits were performed according to the manufacturer's instructions. Briefly, each array kit consists of 4 nitrocellulose membranes with spotted capture and controls antibodies embedded in duplicates, each membrane allowing for 1 urine sample to be assessed (therefore 4 patients per kit). Three sets of each kit (12 membranes) were available, which allowed for the analysis of 12 different patients' urine samples. The assays were conducted by performing 4 membranes at a time per day and subjects were intentionally divided to include at least one of each group in order to reduce selection bias. Due to the semi-quantitative nature of the membranes, we did not assess for intra/inter-variability, but we calculated Pearson's correlation coefficient on the fold changes of the duplicated proteins.

For clarity, the Human Kidney Biomarker Array kit will be further referred to as "Kit K" and the Human XL Cytokine Array kit as "Kit C" throughout the study.

2.3.1. Human Kidney Biomarker Array—Kit K

The nitrocellulose membranes were blocked for 1 h using the provided array buffer and incubated with the capture antibody cocktail and 500 µL of thawed urine at 4 °C overnight on a rocking platform shaker. Urine samples were frozen again pending Kit C analysis. Membranes were then washed, incubated with horseradish peroxidase-conjugated Streptavidin (Streptavidin-HRP) and chemiluminescence reagents. Signal intensity was measured using enhanced chemiluminescence on the ChemiDoc MP Imaging System (Bio-Rad Laboratories Ltd., Watford, UK).

2.3.2. Human XL Cytokine Array Kit—Kit C

Urine samples were thawed and centrifuged at 300× *g* for 5 min. Membranes were blocked for 1 h using the provided array buffer and incubated with 400–450 µL of urine at 4 °C overnight on a rocking platform shaker. Membranes where then washed, incubated with biotinylated detection antibodies, followed by the addition of Streptavidin-HRP and chemiluminescence reagents. Signal intensity was measured using enhanced chemiluminescence on the ChemiDoc MP Imaging System (Bio-Rad Laboratories Ltd., Watford, UK).

2.3.3. Creatinine Quantification

All results were corrected for the urinary creatinine concentration and automated quantification of urinary creatinine was run by the Biochemistry Department (Alder Hey Children's NHS Foundation Trust, Liverpool, UK) using an enzymatic creatinine method [14] with an Alinity Ci System analyser (Abbott, Abbott Park, IL, USA).

2.4. Ethical Approval

All procedures involving human subjects were conducted in accordance with NIHR Good Clinical Practice, HTA Codes of Practice, the Declaration of Helsinki and comparable ethical standards. This study was part of the IgA Vasculitis study which was approved by HRA and Health and Care Research Wales (HCRW) on 21 June 2019 (REC 17/NE/0390, protocol UoL001347, IRAS 236599). Written informed consent was obtained from parents and children prior to any study-related procedure.

2.5. Data Analysis

The intensity of each individual chemiluminescence signal was measured using ImageJ software (NIH) [15] and ImageJ Protein Array Analyser Macro [16] with automatic background removal. To determine the relative concentration of each protein, the average value of the duplicates was measured and normalised to the average of the 6 positive control points, the volume of urine aliquot used and the urinary creatinine concentration. For each protein, fold change was obtained by calculating the ratios of the average relative concentration per group for IgAVN/IgAVwoN, IgAVN/HC and IgAVwoN/HC. Data are presented as the fold change (FCx) between two groups.

2.6. Statistical Analysis

Clinical and demographical data were compared with the Statistical Package for the Social Science (SPSS) version 27.0 software for Windows (IBM Corp, Armonk, NY, USA). Due to the small sample size, data were assumed to be non-normally distributed and Mann–Whitney U/Kruskal–Wallis with Dunn–Bonferroni post hoc tests were used for continuous variables. Pearson's chi-square was applied to categorical variables. For the analytes, multivariate principal component analysis (PCA) was performed using the MetaboAnalyst 5.0 online platform [17] with log2 transformation and pareto scaling. The Student's *t*-test was applied on the log2 transformed data. *p*-Values and fold change (FC) were combined using the "EnhancedVolcano" package [18] in R Statistical Software version 4.1.2 (R Foundation for Statistical Computing, Vienna, Austria) to generate volcano plots and identify significant proteins. A *p*-value of <0.05 was considered statistically significant. Protein concentrations were considered to be elevated for FC $\geq$ 2.0 (2x) and reduced for FC $\leq$ 0.5 (0.5x).

3. Results

3.1. Paediatric Cohort

The study identified 8 children with IgAV from a cohort of 51 recruits to be included in this exploratory analysis. This included four children with nephritis selected as detailed previously and four children without any evidence of past or recent nephritis (termed as 4 IgAVN and 4 IgAVwoN). Four HCs were randomly selected from a cohort of 18 HC children who had been previously recruited to the IgA Vasculitis study. Demographics and baseline characteristics are presented in Table 1. The male:female ratio was 1:1, and the median age was 7.6 years old (range: 4.0–13.4). In those patients with IgAVN, renal involvement was confirmed histologically in all patients (grade II, $n = 1$; grade IIIb, $n = 3$), and the median UACR at the time of sample collection was 542.2 (110.4–2357.7) mg/mmol. All patients within the IgAVN cohort had nephrotic range proteinuria as an indication for conducting a renal biopsy with a mildly reduced serum albumin concentration but no evidence of peripheral oedema. They all had normal blood pressure, normal serum complement titres and normal renal function. The histological analysis demonstrated diffuse proliferative glomerulonephritis with <50% crescents in three patients and focal proliferative glomerulonephritis in one patient. All patients had IgA-positive staining on immunofluorescence (IF) and three out of the four patients had C3 positivity on IF. One patient had patchy evidence of acute tubular inflammation and one patient had <5% interstitial fibrosis. Regarding treatment at the time of sample collection, one IgAVN patient was taking oral prednisolone and one patient was taking both prednisolone and

azathioprine. Subsequent treatments included azathioprine and prednisolone for one patient; mycophenolate moefetil, ACEi and prednisolone for another; ACEi only for the third patient; prednisolone and azathioprine for the fourth patient. One healthy control was prescribed somatotropin at the time of sample collection. None of the IgAVwoN patients received any medication. At 12 months, all of the IgAVwoN patients were discharged and three patients with IgAVN remained under follow up.

Table 1. Patients characteristics at baseline.

	Overall	IgAVN	IgAVwoN	HC	*p*-Value
n	12	4	4	4	-
Male/female	6/6	2/2	2/2	2/2	1.000
Age, years [a]	7.6 [4.0–13.4]	9.9 [5.2–12.2]	5.1 [4.0–6.8]	8.8 [7.5–13.4]	0.058
Time from diagnosis to sample (weeks) [a]	14.8 [1.0–32.0]	14.8 [5.3–25.3]	19.2 [1.0–32.0]	-	0.773
Hypertension [b]	2	2	0	-	0.102
Serum creatinine, mg/dL [a]	42.0 [32.0–54.0]	43.5 [33.0–54.0]	32.0 [32.0–32.0] [c]	-	0.400
Urinary creatinine, mmol/L [a]	7.8 [1.2–19.9]	6.6 [1.6–13.2]	7.8 [1.2–9.4] ± 3.6	9.3 [6.8–19.9]	0.668
UACR, mg/mmol [a,d]	0.0 [0.0–2357.7]	542.2 [110.4–2357.7]	0.0 [0.0–0.0]	0.0 [0.0–0.0]	-
Biopsy proven nephritis [b]	-	4	0	-	-
ISKDC Grade					
II [b]	-	1	-	-	-
IIIb [b]	-	3	-	-	-
Medications [b]	3	2	0	1	-
Corticosteroids [b]	2	Prednisolone (2)	-	-	-
DMARDs [b]	1	Azathioprine (1)	-	-	-
Other [b]	1	-	-	Somatropin (1)	-
Follow up at 12 months					
Discharged	5	1	4	-	
Still under follow up	3	3	-	-	

[a] Median [range]; [b] *n*; [c] serum creatinine only available for one IgAVwoN patient; [d] UACR of 0 mg/mmol assumed if urine dipstick negative for protein. UACR: urinary-albumin-to-creatinine ratio; ISKDC: International Study for Kidney Disease in Children Classification; DMARDs: disease modifying antirheumatic drugs.

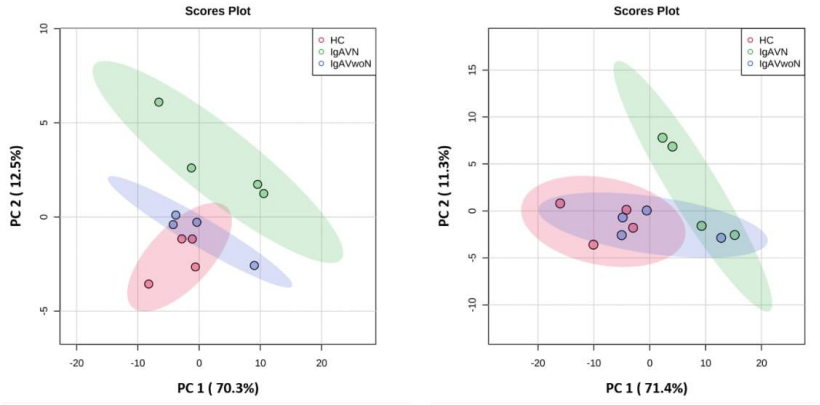
3.2. Exploratory Data Analysis

Principal component (PC) analysis was conducted to visualise the data sets using the first two principal components, and the score plots for each kit are presented in Figure 1a,b (Kit K: PC1 × PC2 = 82.8%; Kit C: PC1 × PC2 = 82.7%). The models demonstrated clear separation between IgAVN and both the IgAVwoN and HC groups. The IgAVN and IgAVwoN clusters demonstrated some areas of overlap in Kit C.

3.3. Comparison between IgAVN and IgAVwoN

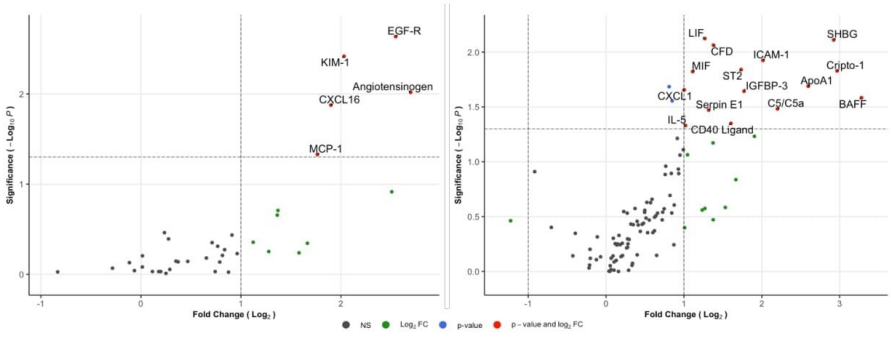
Urinary levels of 20 proteins (5 in Kit K: 3.1% and 15 in Kit C: 14.3%) were significantly increased in IgAVN compared to IgAVwoN (Figure 2a,b). Proteins significant in either IgAVN vs. IgAVwoN or IgAVN vs. HC comparisons are shown in Table 2; full results can be found in Supplementary Materials Tables S2 and S3. The main features identified were B-cell activating factor (BAFF; 9.7x), Cripto-1 (7.8x), sex-hormone-binding globulin (SHBG; 7.6x), angiotensinogen (AGT; 6.5x), apolipoprotein A1 (ApoA1; 6.0x) and epithelial growth factor receptor (EGF-R; 5.8x). One interleukin (i.e., IL-5) and two components of the complement system (i.e., C5/C5a and complement factor D—(CFD)) were also significant, with a fold change of, respectively, 2.0, 4.6 and 2.0. Endoglin (1.75x; $p = 0.021$) and HGF (1.80x; $p = 0.028$) were both statistically significant but did not reach the fold-change

criteria. The urinary concentration of one marker was reduced, but this was not statistically significant (myeloperoxidase; 0.4x; $p = 0.345$).



(**a**) (**b**)

Figure 1. Exploratory data analysis representation: (**a**) Kit K: scores plot between principal component (PC) 1 and PC2 with the explained variance shown in brackets; (**b**) Kit C: scores plot between PC1 and PC2 with the explained variance shown in brackets.



(**a**) (**b**)

Figure 2. Volcano plots of IgAVN vs. IgAVwoN: (**a**) Kit K; (**b**) Kit C. The horizontal lines represent the statistical significance cut off (−log10(0.05)). Vertical lines represent the fold-change cut-off (log2(0.5)−log2(2.0)).

Table 2. A summary of the urinary proteins that were statistically significantly changed in both assays combined as sorted in order of descending fold change (IgAVN vs. IgAVwoN). Bold text represents duplicated proteins that were measured in both kits where results from Kit C are reported. * $p < 0.05$.

Protein	IgAVN vs. IgAVwoN		IgAVN vs. HCs	
	Fold Change	*p*-Value	Fold Change	*p*-Value
BAFF	9.7	0.026 *	26.5	0.004 *
Cripto-1	7.8	0.015 *	7.1	0.026 *
SHBG	7.6	0.008 *	13.3	0.004 *

Table 2. *Cont.*

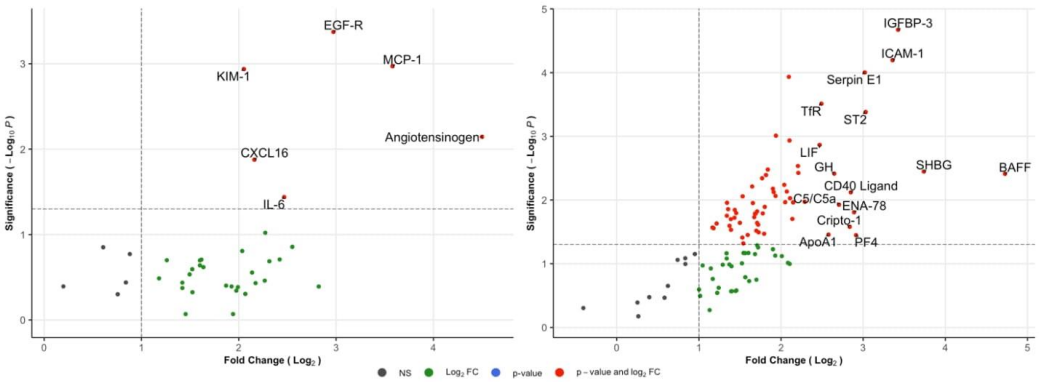
Protein	IgAVN vs. IgAVwoN		IgAVN vs. HCs	
	Fold Change	*p*-Value	Fold Change	*p*-Value
Angiotensinogen	6.5	0.010 *	22.6	0.007 *
ApoA1	6.0	0.020 *	6.0	0.035 *
EGF-R	5.8	0.002 *	7.8	<0.001 *
C5/C5a	4.6	0.033 *	6.5	0.012 *
KIM-1	4.1	0.004 *	4.1	0.001 *
ICAM-1	4.0	0.012 *	10.2	<0.001 *
ENA-78	3.7	0.059	7.4	0.016 *
CXCL16	3.7	0.013 *	4.5	0.013 *
IGFBP-3	3.4	0.023 *	10.7	<0.001 *
MCP-1	3.4	0.047 *	12.0	0.001 *
ST2	3.3	0.014 *	8.2	<0.001 *
CD40 Ligand	3.0	0.045 *	7.2	0.008 *
PF4	2.9	0.261	7.5	0.036 *
CFD	2.6	0.009 *	3.4	0.005 *
GH	2.6	0.067	6.3	0.004 *
IL-6	2.6	0.196	5.5	0.036 *
Serpin E1	2.5	0.034 *	8.1	<0.001 *
LIF	2.4	0.008 *	5.5	0.001 *
MIF	2.2	0.015 *	3.1	0.006 *
Fas Ligand	2.1	0.086	3.8	0.008 *
IL-5	2.0	0.047 *	4.6	0.003 *
CXCL1	2.0	0.022 *	4.3	<0.001 *
IL-4	2.0	0.078	4.6	0.004 *
TfR	1.9	0.087	5.6	<0.001 *
RANTES	1.9	0.128	4.4	0.011 *
IL-8	1.9	0.117	2.9	0.039 *
MCP-1	1.9	0.061	4.1	0.006 *
IL-22	1.8	0.248	4.4	0.020 *
HGF	1.8	0.028 *	4.3	0.001 *
IP-10	1.8	0.128	2.8	0.023 *
IGFBP-2	1.8	0.203	4.9	0.011 *
Endoglin	1.8	0.021 *	2.7	0.014 *
MIG	1.7	0.110	3.8	0.001 *
Flt-3 Ligand	1.7	0.131	2.7	0.019 *
Leptin	1.7	0.268	4.3	0.009 *
IL-10	1.7	0.288	3.3	0.032 *
IL-19	1.5	0.320	3.8	0.009 *
G-CSF	1.5	0.220	3.6	0.003 *
PDGF-AA/BB	1.5	0.236	4.2	0.007 *
TARC	1.5	0.340	4.1	0.011 *
GM-CSF	1.4	0.234	3.2	0.016 *
Angiopoietin-1	1.4	0.326	3.5	0.034 *
MIP-1α/MIP-1β	1.4	0.277	2.6	0.014 *
IL-15	1.4	0.289	3.2	0.011 *
IL-33	1.4	0.363	3.7	0.007 *
IL-31	1.4	0.371	3.3	0.015 *
IL-23	1.4	0.431	3.3	0.024 *
MCP-3	1.3	0.377	3.2	0.016 *
MIP-3α	1.3	0.349	2.5	0.018 *
IL-16	1.3	0.374	2.7	0.016 *
I-TAC	1.2	0.370	2.9	0.009 *
Relaxin-2	1.2	0.510	2.9	0.048 *
IL-24	1.2	0.504	3.2	0.019 *
IL-13	1.2	0.603	3.4	0.016 *
IFN-γ	1.2	0.609	3.2	0.030 *
IL-34	1.2	0.554	2.6	0.025 *

Table 2. *Cont.*

Protein	IgAVN vs. IgAVwoN		IgAVN vs. HCs	
	Fold Change	*p*-Value	Fold Change	*p*-Value
Pentraxin-3	1.1	0.732	3.0	0.035 *
IL-27	1.1	0.570	3.3	0.023 *
TNF-α	1.1	0.469	2.3	0.023 *
Dkk-1	1.1	0.562	2.5	0.011 *
IL-32	1.1	0.558	3.5	0.004 *
BDNF	1.0	0.757	2.6	0.020 *
PDGF-AA	1.0	0.735	2.2	0.027 *
MIP-3b	0.9	0.484	3.5	0.013 *
Thrombospondin-1	0.9	0.781	2.6	0.029 *
TGF-α	0.9	0.929	2.3	0.028 *

3.4. Comparison between IgAVN and the HCs

Analysis of IgAVN versus the HCs revealed 68 proteins with significantly increased urinary concentrations (Figure 3a,b and Table 2; full results in Tables S2 and S3). The highest reported fold change was BAFF (26.5x) followed by angiotensinogen (22.6x), SHBG (13.3x) and MCP-1 (12.0x). Eighteen interleukins out of the 26 assessed by the arrays were significantly elevated compared to the control levels. All of the significant proteins identified by the IgAVN vs. IgAVwoN comparison were also significant in this comparison. No proteins had reduced levels between the two groups.

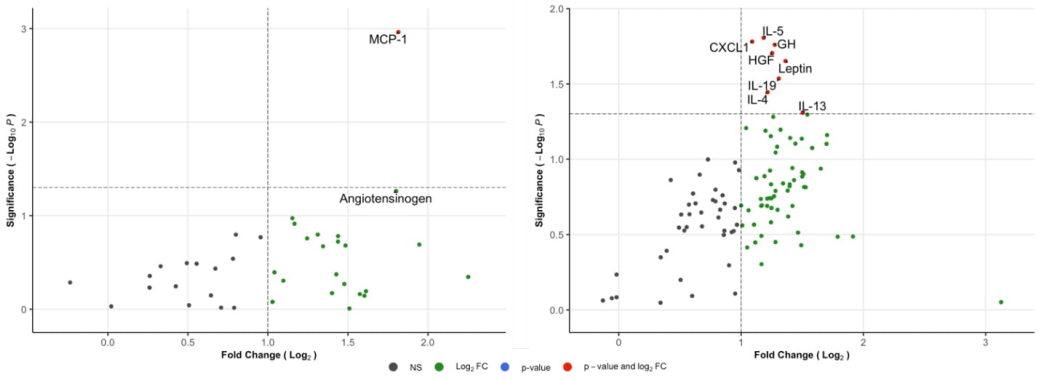


(**a**) (**b**)

Figure 3. Volcano plots of IgAVN vs. IgAVwoN: (**a**) Kit K; (**b**) Kit C. The horizontal lines represent the statistical significance cut off (−log10(0.05)). Vertical lines represent the fold-change cut-off (log2(2.0)). Fifteen top hits in term of FC are labelled in (**b**).

3.5. Comparison between IgAVwoN and the HCs

The concentrations of nine proteins were increased in the urine of IgAVwoN patients when compared to the HCs (Figure 4a,b). MCP-1 levels were statistically significantly increased (3.5x; $p = 0.001$); angiotensinogen was increased, although not reaching statistical significance (3.5x; $p = 0.055$). Urinary IL-13 (2.8x; $p = 0.049$), leptin (2.6x; $p = 0.022$), IL-19 (2.5x; $p = 0.029$), GH (2.4x; $p = 0.017$), HGF (2.4x; $p = 0.020$), IL-4 (2.3x; $p = 0.036$), IL-5 (2.3x; $p = 0.016$) and CXCL1 (2.1x; $p = 0.017$) concentrations were all elevated in IgAVwoN patients compared to the HCs.



(**a**) (**b**)

Figure 4. Volcano plots of IgAVwoN vs. HCs: (**a**) Kit K; (**b**) Kit C. The horizontal lines represent the statistical significance cut-off (−log10(0.05)). Vertical lines represent the fold-change cut-off (log2(2.0)).

3.6. Correlation between the Kit K and C

A total of 19 proteins were assessed in both Kit K and Kit C. Moderate positive correlation [19] (Pearson's correlation coefficient of 0.640) was found between the two kits when comparing the reported average fold changes. MCP-1 reached statistical significance in all comparisons in Kit K as well as in IgAVN vs. IgAVwoN in Kit C and demonstrated a very similar *p*-value, although it was located on either side of the cut-off, for IgAVN vs. IgAVwoN ($p = 0.061$) in Kit C. CXCL1 was found to be significant in all comparisons in Kit C only. The fold changes and *p*-values of six proteins that were reported as statistically significant in at least one kit are reported in Table 3. There was agreement on a further 13 proteins which did not reach statistical significance in both kits.

Table 3. Proteins reported significant by one comparison in at least one kit. Data are presented as the fold change (*p*-value). The bold text highlights discrepancies in the results of significance between the kits.

Protein	IgAVN vs. IgAVwoN		IgAVN vs. HC		IgAVwoN vs. HC	
	Kit K	Kit C	Kit K	Kit C	Kit K	Kit C
CXCL1	**1.8 (0.534)**	**2.0 (0.022)**	**5.0 (0.206)**	**4.3 (<0.001)**	**2.8 (0.539)**	**2.1 (0.017)**
IL-6	2.6 (0.196)	1.8 (0.293)	5.5 (0.036)	3.3 (0.052)	2.1 (0.496)	1.8 (0.197)
IL-10	1.6 (0.659)	1.7 (0.288)	**4.8 (0.346)**	**3.3 (0.032)**	3.1 (0.642)	2.0 (0.203)
MCP-1	3.4 (0.047)	1.9 (0.061)	12.0 (0.001)	4.1 (0.006)	**3.5 (0.001)**	**2.2 (0.134)**
Thrombospondin-1	1.4 (0.719)	0.9 (0.781)	**3.7 (0.397)**	**2.6 (0.029)**	2.6 (0.673)	2.9 (0.153)
TNF-α	1.6 (0.446)	1.1 (0.469)	**2.7 (0.366)**	**2.3 (0.023)**	1.6 (0.963)	2.1 (0.385)

4. Discussion

This study aimed to use an exploratory small cohort to assess the relative concentrations of 124 known urine markers of kidney inflammation and injury in children with IgAV to identify differences between those with and without established renal involvement to provide insight into pathophysiological mechanisms. The demographic data, renal parameters and histological features of our cohort were typical of how the majority of children present with IgAVN, and we report increased relative urinary levels of 20 proteins in IgAVN when compared to IgAVwoN. To our knowledge, this exploratory study is the first to evaluate such an extensive panel of urinary proteins in children with IgAVN. Although the study cohort was limited by size, we report some novel findings that provide new insight into the pathophysiology of IgAVN.

A recognised feature of IgA-related renal diseases is the presence of galactose deficient IgA, known as Gd-IgA1, and glycan-specific IgG and IgA antibodies, which are found at increased circulating levels in IgAVN [11]. They are mainly derived from activated B-cell lymphocytes, and effective B-cell maturation relies upon B-cell activating factor (BAFF) [20]. In our study, urinary BAFF levels increased by 9.7-fold between IgAVN and IgAVwoN and by 26.5-fold between IgAVN and HCs. The role of BAFF in IgAV remains unclear, as it has only been explored in one study that did not find any association between BAFF gene polymorphisms and IgAV [21]. BAFF is a cytokine of the tumour necrosis factor (TNF) family, and some evidence suggests it could activate the tumour necrosis receptor-associated factor 6 (TRAF6)/NF-kB pathway in glomerular mesangial cells, consequently contributing to the pathogenesis of IgA nephropathy [22]. Vincent and colleagues were unable to detect urinary BAFF in adult patients with IgA nephropathy [23]; however, in mice, overexpression of BAFF resulted in an IgA nephropathy–renal phenotype and hyper-IgA syndrome [24,25]. In addition to IgA nephropathy, raised serum BAFF levels have also been linked to other autoimmune diseases such as systemic lupus erythematosus (SLE) and multiple sclerosis [20]. Increased urinary BAFF levels and its usefulness as a biomarker has been reported in lupus nephritis [23,26]. Our data suggest that BAFF could be involved in the pathophysiology of IgAVN, and this warrants further analysis in validation studies.

One of the other significantly elevated proteins in this study was Cripto-1, an embryonic protein that is re-expressed during inflammation, wound healing and tumorigenesis [27]. Several in vitro studies have shown that Cripto-1 modulates macrophage cytokine secretion, resulting in elevated concentrations of pro-inflammatory cytokines TNF-α, IL-6, IL-1β and anti-inflammatory cytokine IL-10 [27,28], all of which have also been linked to the pathogenesis of IgAV and IgAVN [29]. In addition, overexpression of Cripto-1 has been reported in other diseases, including Crohn's disease, ulcerative colitis and rheumatoid arthritis [27], suggesting a link to autoinflammatory processes. Increased urinary concentrations of Cripto-1 in our IgAVN cohort supports a potential role of this modulating protein in IgAVN.

The potential biological significance of our finding of increased urinary sex-hormone-binding globulin (SHBG) levels in IgAVN is unclear. SHBG is a glycoprotein primarily synthesised by the liver, and it is involved in transporting androgen and oestrogen. SHBG serum levels are stable in childhood and decrease at puberty, especially in boys [30]. In vitro, SHBG suppresses inflammation in macrophages [31], and it is downregulated by TNF-α [32] and IL-1β [33]. In our cohort, two of the IgAVN patients were taking prednisolone, and the pubertal status of our cohort was not documented. The impact of synthetic glucocorticoids on urinary SHBH is unknown, although some studies have reported lowered SHBG serum levels for patients undergoing treatment with corticosteroids [34].

The significantly increased concentration of urinary angiotensinogen in this study is not too surprising, because extensive evidence supports the existence of an intrarenal RAAS system that contributes to kidney disease progression [35], and the benefits of RAAS inhibition, as a renoprotective treatment, is well recognised for glomerular diseases [36]. Furthermore, the RAAS system may have a direct role in complement pathway activation through renin-mediated C3 cleavage as demonstrated in vitro and suggested through a study evaluating the efficacy of direct renin inhibition (aliskiren) in dense deposit disease where reduced systemic and renal complement activation was observed [37]. ACE gene polymorphisms have also been associated with IgAV susceptibility [38]. The majority of the circulating AGT is produced by the liver but local renal production by the proximal tubules has been demonstrated in animal models [39]. In a study by Nishiyama and colleagues, urinary AGT (UAGT) levels were raised in patients with IgA nephropathy, and this was shown to correlate with renal tissue gene expression of AGT and angiotensin II immunoreactivity. Thus, UAGT was proposed to provide a specific index of intrarenal RAAS activity in patients with IgA nephropathy [40]. We found that urinary UAGT concentrations increased by 6.5-fold in IgAVN compared to IgAVwoN, and this was observed by two previous studies that found remarkably elevated UAGT levels in children with

IgAVN [41,42]. However, urinary renin levels were not significantly different across the groups in the current study. Interestingly, in our cohort, UAGT levels were also increased in patients without nephritis, although this finding was not statistically significant ($p = 0.055$). This could be due to the fact of a spurious finding, but other possible explanations include the relationship of the RAAS system with systemic vascular inflammation [43] and/or the presence of subclinical kidney inflammation not detectable using current methods of disease activity monitoring.

We report elevated levels of urinary apolipoprotein A1 (ApoA1), which is a novel finding in IgAVN. ApoA1 is the main protein component of high-density lipoproteins (HDL) [44], and although HDL has a protective role in cardiovascular disease and it has been widely studied, more recent evidence suggests HDL may contribute to kidney disease [45] and autoimmunity [46]. A recent study reported elevated urinary ApoA1 in children with various kidney diseases (including 18 children with glomerulonephritis) [47], whilst children with nephrotic syndrome in remission, nephrolithiasis, polycystic kidney disease, transplant or hypertension did not show higher ApoA1 levels [47]. Identifying whether ApoA1 is excreted in a modified form and its effect on downstream lipid pathways could provide more information on the renal handling of lipoproteins and their role in this disease.

Activation of both the alternative and mannose-binding lectin complement pathways have been demonstrated in IgAV [11], with skin and mesangial deposits reported to contain complement components C3 and C5b-9 [48,49]. In addition, C4d and C5b-9 positivity in renal histology has been associated with poor renal outcomes in patients with IgAVN and IgA nephropathy [50]. The findings of increased C5/C5a and complement factor D concentrations in the urine of children with IgAVN compared to IgAVwoN (fold changes of, respectively, 4.6 and 2.6) raises the potential role of the complement system in the pathophysiology of this condition and supports this pathway as a possible treatment target. C5 cleaves into C5a—a potent neutrophil chemoattractant—and C5b, which ultimately leads to the formation of the membrane attack complex (MAC) [51]. C5a has also been shown to increase production of IL-8, MCP-1 and ICAM-1 by endothelial cells in vitro [48], and these markers were also increased in our cohort. Further confirmation regarding the role of the complement pathway in IgAVN is warranted.

Our findings are consistent with those reported in a recent systematic literature review that identified urinary biomarkers in children with IgAVN [12]. The current summary of the literature supported the significance of UAGT, β2-microglobulin, KIM-1 and MCP-1 [12]. In addition, individual studies have reported elevated urinary levels of MIF [52], MMP-9 [53], IL-6, IL-8 and IL-10 [54] in paediatric IgAVN. Our study did not find elevated levels of β2-microglobulin, MMP-9 and IL-6/8/10 in IgAVN; however, it is important to note that this was a small preliminary study using a semi-quantitative technique. Dyga and colleagues did not find any significant association between the concentrations of urinary NGAL and L-FABP [55], which is in keeping with our findings. Our findings of elevated urinary proteins, which could result from tubulointerstitial inflammation/injury (KIM-1 [56], ApoA1 [47,57], EGF-R [58], AGT [59]), further support that IgAVN includes some degree of tubular inflammation in addition to the glomerulonephritis as previously proposed by Williams et al. [12]. Finally, it remains unclear whether these markers are specific to IgAVN or result from pathophysiological features shared between different renal diseases, such as excessive inflammation, or a by-product of proteinuria. While this study was limited in terms of sample size, it provides exploratory data to direct future studies, and it demonstrates that urinary protein profiles do vary between IgAV and IgAVN patients. Future work will look to utilise more robust quantitative methods, such as ELISAs and LC-MS/MS, to validate the findings of the most highly expressed proteins using a larger patient cohort.

5. Conclusions

The current study utilised a small number of patients in a cross-sectional design to explore a large number of urinary proteins in patients with established IgAVN. It was able to uncover some interesting variations in the urinary protein profiles that may help elucidate further mechanisms of pathophysiology and represent potential drug targets for intervention. However, there are limitations to this study, predominantly related to the sample size, and these findings require validation using larger cross-sectional cohorts. Furthermore, longitudinal evaluation would allow for the evolution of nephritis to be better characterised, which would significantly improve our mechanistic understanding of the development of IgAVN.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/children9050622/s1, Table S1: Proteins assessed by R&D's Systems Human Kidney Biomarker Array Kit ("Kit K") and the Human XL Cytokine Array Kit ("Kit C"); Table S2: Full results—Human Kidney Biomarker Array Kit; Table S3: Full results—Human XL Cytokine Array Kit.

Author Contributions: Conceptualisation, R.D.W. and L.O.; methodology, R.D.W., J.M. and L.O.; formal analysis, J.M. and A.J.C.; investigation, J.M. and R.D.W.; resources, S.D., L.O., J.M., R.D.W. and A.J.C.; writing—original draft preparation, J.M. and L.O.; writing—review and editing, J.M., L.O., A.J.C., R.D.W. and S.D.; visualisation, J.M.; supervision, L.O.; project administration, L.O.; funding acquisition, L.O. and R.D.W. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Funding Autoimmune Research (F.A.I.R.) Charity (Registered U.K. Charity; number: 1176388).

Institutional Review Board Statement: All procedures involving human subjects were conducted in accordance with NIHR Good Clinical Practice, HTA Codes of Practice, the Declaration of Helsinki and comparable ethical standards. This study was part of the IgA Vasculitis study, which was approved by HRA and Health and Care Research Wales (HCRW) on 21 June 2019, REC 17/NE/0390, protocol UoL001347, IRAS 236599.

Informed Consent Statement: Written informed consent was obtained from parents and children prior to any study-related procedure.

Data Availability Statement: The data presented in this study are available in the Supplementary Materials Tables S2 and S3.

Acknowledgments: The authors thank the volunteers who participated in the study. This work was further supported by the UK's Experimental Arthritis Treatment Centre for Children (supported by Versus Arthritis, Alder Hey Children's NHS Foundation Trust, the Alder Hey Charity and the University of Liverpool) and partially carried out at the NIHR Alder Hey Clinical Research Facility. We specifically thank Catherine McBurney (NIHR Alder Hey Clinical Research Facility) for her work in recruiting patients to the IgA Vasculitis study. We thank Andrew Hodgkinson and the Biochemistry Department, Alder Hey Children's NHS Foundation Trust, for performing the urinary creatinine quantification. We thank Amandine Charras and Francesca Sposito (Department of Women's and Children's Health, University of Liverpool) for kindly sharing their expertise in data analysis. The views expressed are those of the authors and not necessarily those of the NHS, the NIHR or the Department of Health and Social Care.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

References

1. Sunderkötter, C.; Lamprecht, P.; Mahr, A.; Metze, D.; Zelger, B. Nomenclature of cutaneous vasculitides—German translation of the dermatologic addendum to the 2012 Revised International Chapel Hill Consensus Conference Nomenclature of Vasculitides. *J. Dtsch. Dermatol. Ges.* **2018**, *16*, 1425–1432. [CrossRef] [PubMed]

2. Gardner-Medwin, J.M.; Dolezalova, P.; Cummins, C.; Southwood, T.R. Incidence of Henoch-Schönlein purpura, Kawasaki disease, and rare vasculitides in children of different ethnic origins. *Lancet* **2002**, *360*, 1197–1202. [CrossRef]
3. Ozen, S.; Pistorio, A.; Iusan, S.M.; Bakkaloglu, A.; Herlin, T.; Brik, R.; Buoncompagni, A.; Lazar, C.; Bilge, I.; Uziel, Y.; et al. EULAR/PRINTO/PRES criteria for Henoch–Schönlein purpura, childhood polyarteritis nodosa, childhood Wegener granulomatosis and childhood Takayasu arteritis: Ankara 2008. Part II: Final classification criteria. *Ann. Rheum. Dis.* **2010**, *69*, 798. [CrossRef]
4. Oni, L.; Sampath, S. Childhood IgA Vasculitis (Henoch Schonlein Purpura)-Advances and Knowledge Gaps. *Front. Pediatr.* **2019**, *7*, 257. [CrossRef] [PubMed]
5. Ozen, S.; Marks, S.D.; Brogan, P.; Groot, N.; de Graeff, N.; Avcin, T.; Bader-Meunier, B.; Dolezalova, P.; Feldman, B.M.; Kone-Paut, I.; et al. European consensus-based recommendations for diagnosis and treatment of immunoglobulin A vasculitis—the SHARE initiative. *Rheumatology* **2019**, *58*, 1607–1616. [CrossRef] [PubMed]
6. Chan, H.; Tang, Y.L.; Lv, X.H.; Zhang, G.F.; Wang, M.; Yang, H.P.; Li, Q. Risk Factors Associated with Renal Involvement in Childhood Henoch-Schönlein Purpura: A Meta-Analysis. *PLoS ONE* **2016**, *11*, e0167346. [CrossRef]
7. Nikibakhsh, A.A.; Mahmoodzadeh, H.; Karamyyar, M.; Hejazi, S.; Noroozi, M.; Macooie, A.A. Treatment of severe henoch-schonlein purpura nephritis with mycophenolate mofetil. *Saudi J. Kidney Dis. Transpl.* **2014**, *25*, 858–863. [CrossRef]
8. Shi, D.; Chan, H.; Yang, X.; Zhang, G.; Yang, H.; Wang, M.; Li, Q. Risk factors associated with IgA vasculitis with nephritis (Henoch-Schönlein purpura nephritis) progressing to unfavorable outcomes: A meta-analysis. *PLoS ONE* **2019**, *14*, e0223218. [CrossRef]
9. Narchi, H. Risk of long term renal impairment and duration of follow up recommended for Henoch-Schonlein purpura with normal or minimal urinary findings: A systematic review. *Arch. Dis. Child.* **2005**, *90*, 916–920. [CrossRef]
10. Watson, L.; Richardson, A.R.; Holt, R.C.; Jones, C.A.; Beresford, M.W. Henoch schonlein purpura–a 5-year review and proposed pathway. *PLoS ONE* **2012**, *7*, e29512. [CrossRef]
11. Song, Y.; Huang, X.; Yu, G.; Qiao, J.; Cheng, J.; Wu, J.; Chen, J. Pathogenesis of IgA Vasculitis: An Up-To-Date Review. *Front. Immunol.* **2021**, *12*, 771619. [CrossRef] [PubMed]
12. Williams, C.E.C.; Toner, A.; Wright, R.D.; Oni, L. A systematic review of urine biomarkers in children with IgA vasculitis nephritis. *Pediatr. Nephrol.* **2021**, *36*, 3033–3044. [CrossRef]
13. Lurbe, E.; Agabiti-Rosei, E.; Cruickshank, J.K.; Dominiczak, A.; Erdine, S.; Hirth, A.; Invitti, C.; Litwin, M.; Mancia, G.; Pall, D.; et al. 2016 European Society of Hypertension guidelines for the management of high blood pressure in children and adolescents. *J. Hypertens.* **2016**, *34*, 1887–1920. [CrossRef] [PubMed]
14. Asano, K.; Iwasaki, H.; Fujimura, K.; Ikeda, M.; Sugimoto, Y.; Matsubara, A.; Yano, K.; Irisawa, H.; Kono, F.; Kanbe, M.; et al. Automated microanalysis of creatinine by coupled enzyme reactions. *Hiroshima J. Med. Sci.* **1992**, *41*, 1–5. [PubMed]
15. Schneider, C.A.; Rasband, W.S.; Eliceiri, K.W. NIH Image to ImageJ: 25 years of image analysis. *Nat. Methods* **2012**, *9*, 671–675. [CrossRef]
16. Carpentier, G.; Henault, E. Protein array analyzer for ImageJ. In Proceedings of the ImageJ User and Developer Conference 2010, Mondorf-les-Bains, Luxembourg, 27–29 October 2010; pp. 238–240.
17. Pang, Z.; Chong, J.; Zhou, G.; de Lima Morais, D.A.; Chang, L.; Barrette, M.; Gauthier, C.; Jacques, P.-É.; Li, S.; Xia, J. MetaboAnalyst 5.0: Narrowing the gap between raw spectra and functional insights. *Nucleic Acids Res.* **2021**, *49*, W388–W396. [CrossRef]
18. Blighe, K.; Rana, S.; Lewis, M. EnhancedVolcano: Publication-Ready Volcano Plots with Enhanced Colouring and Labeling. 2018. Available online: https://github.com/kevinblighe/EnhancedVolcano (accessed on 12 February 2022).
19. Mukaka, M.M. Statistics corner: A guide to appropriate use of correlation coefficient in medical research. *Malawi Med. J.* **2012**, *24*, 69–71.
20. Moisini, I.; Davidson, A. BAFF: A local and systemic target in autoimmune diseases. *Clin. Exp. Immunol.* **2009**, *158*, 155–163. [CrossRef]
21. Prieto-Peña, D.; Genre, F.; Remuzgo-Martínez, S.; Pulito-Cueto, V.; Atienza-Mateo, B.; Llorca, J.; Sevilla-Pérez, B.; Ortego-Centeno, N.; Lera-Gómez, L.; Leonardo, M.T.; et al. BAFF, APRIL and BAFFR on the pathogenesis of Immunoglobulin-A vasculitis. *Sci. Rep.* **2021**, *11*, 11510. [CrossRef]
22. Cao, Y.; Lu, G.; Chen, X.; Chen, X.; Guo, N.; Li, W. BAFF is involved in the pathogenesis of IgA nephropathy by activating the TRAF6/NF-κB signaling pathway in glomerular mesangial cells. *Mol. Med. Rep.* **2020**, *21*, 795–805. [CrossRef]
23. Vincent, F.B.; Kandane-Rathnayake, R.; Hoi, A.Y.; Slavin, L.; Godsell, J.D.; Kitching, A.R.; Harris, J.; Nelson, C.L.; Jenkins, A.J.; Chrysostomou, A.; et al. Urinary B-cell-activating factor of the tumour necrosis factor family (BAFF) in systemic lupus erythematosus. *Lupus* **2018**, *27*, 2029–2040. [CrossRef] [PubMed]
24. McCarthy, D.D.; Chiu, S.; Gao, Y.; Summers-deLuca, L.E.; Gommerman, J.L. BAFF induces a hyper-IgA syndrome in the intestinal lamina propria concomitant with IgA deposition in the kidney independent of LIGHT. *Cell. Immunol.* **2006**, *241*, 85–94. [CrossRef] [PubMed]
25. McCarthy, D.D.; Kujawa, J.; Wilson, C.; Papandile, A.; Poreci, U.; Porfilio, E.A.; Ward, L.; Lawson, M.A.; Macpherson, A.J.; McCoy, K.D.; et al. Mice overexpressing BAFF develop a commensal flora-dependent, IgA-associated nephropathy. *J. Clin. Investig.* **2011**, *121*, 3991–4002. [CrossRef]

26. Phatak, S.; Chaurasia, S.; Mishra, S.K.; Gupta, R.; Agrawal, V.; Aggarwal, A.; Misra, R. Urinary B cell activating factor (BAFF) and a proliferation-inducing ligand (APRIL): Potential biomarkers of active lupus nephritis. *Clin. Exp. Immunol.* **2017**, *187*, 376–382. [CrossRef]
27. Klauzinska, M.; Bertolette, D.; Tippireddy, S.; Strizzi, L.; Gray, P.C.; Gonzales, M.; Duroux, M.; Ruvo, M.; Wechselberger, C.; Castro, N.P.; et al. Cripto-1: An extracellular protein—connecting the sequestered biological dots. *Connect. Tissue Res.* **2015**, *56*, 364–380. [CrossRef] [PubMed]
28. Zhang, D.-m.; Bao, Y.-L.; Yu, C.-L.; Wang, Y.-m.; Song, Z.-B. Cripto-1 modulates macrophage cytokine secretion and phagocytic activity via NF-κB signaling. *Immunol. Res.* **2016**, *64*, 104–114. [CrossRef]
29. Sugino, H.; Sawada, Y.; Nakamura, M. IgA Vasculitis: Etiology, Treatment, Biomarkers and Epigenetic Changes. *Int. J. Mol. Sci.* **2021**, *22*, 7538. [CrossRef]
30. Aydın, B.; Winters, S.J. Sex Hormone-Binding Globulin in Children and Adolescents. *J. Clin. Res. Pediatr. Endocrinol.* **2016**, *8*, 1–12. [CrossRef]
31. Yamazaki, H.; Kushiyama, A.; Sakoda, H.; Fujishiro, M.; Yamamotoya, T.; Nakatsu, Y.; Kikuchi, T.; Kaneko, S.; Tanaka, H.; Asano, T. Protective Effect of Sex Hormone-Binding Globulin against Metabolic Syndrome: In Vitro Evidence Showing Anti-Inflammatory and Lipolytic Effects on Adipocytes and Macrophages. *Mediat. Inflamm.* **2018**, *2018*, 3062319. [CrossRef]
32. Simó, R.; Barbosa-Desongles, A.; Sáez-Lopez, C.; Lecube, A.; Hernandez, C.; Selva, D.M. Molecular Mechanism of TNFα-Induced Down-Regulation of SHBG Expression. *Mol. Endocrinol.* **2012**, *26*, 438–446. [CrossRef]
33. Simó, R.; Barbosa-Desongles, A.; Hernandez, C.; Selva, D.M. IL1β down-regulation of sex hormone-binding globulin production by decreasing HNF-4α via MEK-1/2 and JNK MAPK pathways. *Mol. Endocrinol.* **2012**, *26*, 1917–1927. [CrossRef] [PubMed]
34. Pugeat, M.; Nader, N.; Hogeveen, K.; Raverot, G.; Déchaud, H.; Grenot, C. Sex hormone-binding globulin gene expression in the liver: Drugs and the metabolic syndrome. *Mol. Cell. Endocrinol.* **2010**, *316*, 53–59. [CrossRef] [PubMed]
35. Urushihara, M.; Kagami, S. Role of the intrarenal renin–angiotensin system in the progression of renal disease. *Pediatr. Nephrol.* **2017**, *32*, 1471–1479. [CrossRef] [PubMed]
36. Inker, L.A.; Astor, B.C.; Fox, C.H.; Isakova, T.; Lash, J.P.; Peralta, C.A.; Kurella Tamura, M.; Feldman, H.I. KDOQI US Commentary on the 2012 KDIGO Clinical Practice Guideline for the Evaluation and Management of CKD. *Am. J. Kidney Dis.* **2014**, *63*, 713–735. [CrossRef]
37. Békássy, Z.D.; Kristoffersson, A.-C.; Rebetz, J.; Tati, R.; Olin, A.I.; Karpman, D. Aliskiren inhibits renin-mediated complement activation. *Kidney Int.* **2018**, *94*, 689–700. [CrossRef]
38. Lee, Y.H.; Choi, S.J.; Ji, J.D.; Song, G.G. Associations between the angiotensin-converting enzyme insertion/deletion polymorphism and susceptibility to vasculitis: A meta-analysis. *J. Renin Angiotensin Aldosterone Syst.* **2012**, *13*, 196–201. [CrossRef]
39. Lantelme, P.; Rohrwasser, A.; Gociman, B.; Hillas, E.; Cheng, T.; Petty, G.; Thomas, J.; Xiao, S.; Ishigami, T.; Herrmann, T.; et al. Effects of dietary sodium and genetic background on angiotensinogen and Renin in mouse. *Hypertension* **2002**, *39*, 1007–1014. [CrossRef]
40. Nishiyama, A.; Konishi, Y.; Ohashi, N.; Morikawa, T.; Urushihara, M.; Maeda, I.; Hamada, M.; Kishida, M.; Hitomi, H.; Shirahashi, N.; et al. Urinary angiotensinogen reflects the activity of intrarenal renin-angiotensin system in patients with IgA nephropathy. *Nephrol. Dial. Transpl.* **2011**, *26*, 170–177. [CrossRef]
41. Yi-fei, M.; Yu-feng, L.; Gui-mei, G.; Ya-ju, Z.; Ying-liang, G.; Yu, D. Changes of new urinary biomarkers in children with Henoch-Schonlein purpura nephritis. *J. Shanghai Jiaotong Univ. Med. Sci.* **2020**, *40*, 841.
42. Mao, Y.N.; Liu, W.E.I.; Li, Y.G.; Jia, G.C.; Zhang, Z.; Guan, Y.J.; Zhou, X.F.; Liu, Y.F. Urinary angiotensinogen levels in relation to renal involvement of Henoch-Schonlein purpura in children. *Nephrology* **2012**, *17*, 53–57. [CrossRef]
43. Pacurari, M.; Kafoury, R.; Tchounwou, P.B.; Ndebele, K. The Renin-Angiotensin-aldosterone system in vascular inflammation and remodeling. *Int. J. Inflam.* **2014**, *2014*, 689360. [CrossRef] [PubMed]
44. Van der Vorst, E.P.C. High-Density Lipoproteins and Apolipoprotein A1. In *Vertebrate and Invertebrate Respiratory Proteins, Lipoproteins and Other Body Fluid Proteins*; Hoeger, U., Harris, J.R., Eds.; Springer International Publishing: Cham, Switzerland, 2020; pp. 399–420.
45. Kon, V.; Yang, H.-C.; Smith, L.E.; Vickers, K.C.; Linton, M.F. High-Density Lipoproteins in Kidney Disease. *Int. J. Mol. Sci.* **2021**, *22*, 8201. [CrossRef] [PubMed]
46. Georgila, K.; Vyrla, D.; Drakos, E. Apolipoprotein A-I (ApoA-I), Immunity, Inflammation and Cancer. *Cancers* **2019**, *11*, 1097. [CrossRef] [PubMed]
47. Clark, A.J.; Jabs, K.; Hunley, T.E.; Jones, D.P.; VanDeVoorde, R.G.; Anderson, C.; Du, L.; Zhong, J.; Fogo, A.B.; Yang, H.; et al. Urinary apolipoprotein AI in children with kidney disease. *Pediatr. Nephrol. Berl. Ger.* **2019**, *34*, 2351–2360. [CrossRef]
48. Yang, Y.-H.; Tsai, I.J.; Chang, C.-J.; Chuang, Y.-H.; Hsu, H.-Y.; Chiang, B.-L. The Interaction between Circulating Complement Proteins and Cutaneous Microvascular Endothelial Cells in the Development of Childhood Henoch-Schönlein Purpura. *PLoS ONE* **2015**, *10*, e0120411. [CrossRef]
49. Davin, J.-C.; Ten Berge, I.J.; Weening, J.J. What is the difference between IgA nephropathy and Henoch-Schönlein purpura nephritis? *Kidney Int.* **2001**, *59*, 823–834. [CrossRef]
50. Chua, J.S.; Zandbergen, M.; Wolterbeek, R.; Baelde, H.J.; van Es, L.A.; de Fijter, J.W.; Bruijn, J.A.; Bajema, I.M. Complement-mediated microangiopathy in IgA nephropathy and IgA vasculitis with nephritis. *Mod. Pathol.* **2019**, *32*, 1147–1157. [CrossRef]
51. Guo, R.F.; Ward, P.A. Role of C5a in inflammatory responses. *Annu. Rev. Immunol.* **2005**, *23*, 821–852. [CrossRef]

52. Wang, J.; Li, Y.; Chen, Y.; Dai, X.; Di, Y.; Shen, M.; Ying, Q.; Fu, S.; Li, Y. Urinary Macrophage Migration Inhibitory Factor as a Noninvasive Biomarker in Pediatric Henoch-Schönlein Purpura Nephritis. *J. Clin. Rheumatol.* **2017**, *23*, 258–261. [CrossRef]
53. Qin, Y.-H.; Zhou, T.-B.; Lei, F.-Y.; Huang, W.-F.; Zhao, Y.-J.; Lin, F.-Q.; Su, L.-N. Cut-off values for serum matrix metalloproteinase-9: Is there a threshold to predict renal involvement for Henoch–Schonlein purpura in children? *Nephrology* **2011**, *16*, 93–99. [CrossRef]
54. Pillebout, E.; Jamin, A.; Ayari, H.; Housset, P.; Pierre, M.; Sauvaget, V.; Viglietti, D.; Deschenes, G.; Monteiro, R.C.; Berthelot, L.; et al. Biomarkers of IgA vasculitis nephritis in children. *PLoS ONE* **2017**, *12*, e0188718. [CrossRef] [PubMed]
55. Dyga, K.; Machura, E.; Świętochowska, E.; Szczepańska, M. Analysis of the association between kidney injury biomarkers concentration and nephritis in immunoglobulin A vasculitis: A pediatric cohort study. *Int. J. Rheum. Dis.* **2020**, *23*, 1184–1193. [CrossRef] [PubMed]
56. Song, J.; Yu, J.; Prayogo, G.W.; Cao, W.; Wu, Y.; Jia, Z.; Zhang, A. Understanding kidney injury molecule 1: A novel immune factor in kidney pathophysiology. *Am. J. Transl. Res.* **2019**, *11*, 1219–1229. [PubMed]
57. Nielsen, R.; Christensen, E.I.; Birn, H. Megalin and cubilin in proximal tubule protein reabsorption: From experimental models to human disease. *Kidney Int.* **2016**, *89*, 58–67. [CrossRef]
58. Zeng, F.; Singh, A.B.; Harris, R.C. The role of the EGF family of ligands and receptors in renal development, physiology and pathophysiology. *Exp. Cell Res.* **2009**, *315*, 602–610. [CrossRef] [PubMed]
59. Ramkumar, N.; Kohan, D.E. Proximal tubule angiotensinogen modulation of arterial pressure. *Curr. Opin. Nephrol. Hypertens.* **2013**, *22*, 32–36. [CrossRef] [PubMed]

8.2. **Appendix 2**

New version of the case report form (CRF) developed this year to collect the demographic and clinical data of participants to the IgAV study (IgAV cohort).

Patient study number:	A	H	V				The IgA vasculitis Study case report form

Consent date: ___/___/_____

Demographics

Date of birth		Postcode	
Gender	☐ Male / ☐ Female	Age at first diagnosis	_____ years_____months

Ethnicity

White	*Mixed or Multiple ethnic groups*	*Asian or Asian British*	*Black, African, Caribbean or Black British*	*Other ethnic group*
☐ English, Welsh, Scottish, Northern Irish or British	☐ White and Black Caribbean	☐ Indian	☐ African	☐ Arab
☐ Irish	☐ White and Black African	☐ Pakistani	☐ Caribbean	☐ Any other ethnic group
☐ Gypsy or Irish Traveller	☐ White and Asian	☐ Bangladeshi	☐ Any other Black, African or Caribbean background	
☐ Any other White background	☐ Any other Mixed or Multiple ethnic background	☐ Chinese		
		☐ Any other Asian background		

First presentation

Date of disease onset ___/___/_____ *Date of diagnosis* ___/___/_____	*Presentation to* ☐ Alder Hey ___/___/____ ☐ GP ___/___/____ ☐ Other _____ ___/___/_____

Clinical features	*Possible triggers*	*Relevant Family History*
☐ Purpuric/petechial rash ___/___/_____ ☐ Abdominal pain ___/___/_____ ☐ Renal involvement ___/___/_____ ☐ Sore or swollen joints ___/___/_____	☐ Preceding sore throat ☐ Preceding viral illness ☐ Preceding bacterial illness ☐ History of recurrent tonsillitis ☐ Dental decay or caries ☐ Any previous dental extraction ☐ Attends the dentist regularly	*Relevant Past Medical History*

Dipstick on Date of Presentation _____/_____/_____ Non-available ☐	*Blood-pressure*
Protein: +/- T 1 2 3 Alb:Cr Ratio _____ Other: Blood: +/- T 1 2 3 Cr _____ Alb _____	/ Height _____ Weight _____

Details

Prior to recruitment

Abnormal Blood Results on Presentation Date: ___/___/___ All within Range ☐ Non-available ☐

Disease monitoring – visits *only list abnormal values for other bloods (ie FBC/ UEs)*

Date	Urine	BP	Blood	Sample	At time of sample	Comments*
___/___/_____ _____ post diagnosis	Protein: +/- T 1 2 3 Blood: +/- T 1 2 3 Alb:Cr Ratio _____ Cr _____ Alb _____	/ Height _____ Weight _____	Cr _____ Alb _____	☐ Urine ☐ Blood ☐ Saliva Episode (lab) _____	☐ HSP rash ☐ Abdominal pain ☐ Joint involvement ☐ Renal involv. ☐ Other ☐ Any medications (incl OTC) in the last 48 hours, list below:	
___/___/_____ _____ post diagnosis	Protein: +/- T 1 2 3 Blood: +/- T 1 2 3 Alb:Cr Ratio _____ Cr _____ Alb _____	/ Height _____ Weight _____	Cr _____ Alb _____	☐ Urine ☐ Blood ☐ Saliva Episode (lab) _____	☐ Rash ☐ Abdominal pain ☐ Joint involvement ☐ Renal involv. ☐ Other ☐ Any medications (incl OTC) in the last 48 hours, list below:	
____/___/_____ _____ post diagnosis	Protein: +/- T 1 2 3 Blood: +/- T 1 2 3 Alb:Cr Ratio _____ Cr _____ Alb _____	/ Height _____ Weight _____	Cr _____ Alb _____	☐ Urine ☐ Blood ☐ Saliva Episode (lab) _____	☐ Rash ☐ Abdominal pain ☐ Joint involvement ☐ Renal involv. ☐ Other ☐ Any medications (incl OTC) in the last 48 hours, list below:	
___/___/_____ _____ post diagnosis	Protein: +/- T 1 2 3 Blood: +/- T 1 2 3 Alb:Cr Ratio _____ Cr _____ Alb _____	/ Height _____ Weight _____	Cr _____ Alb _____	☐ Urine ☐ Blood ☐ Saliva Episode (lab) _____	☐ Rash ☐ Abdominal pain ☐ Joint involvement ☐ Renal involv. ☐ Other ☐ Any medications (incl OTC) in the last 48 hours, list below:	
___/___/_____ _____ post diagnosis	Protein: +/- T 1 2 3 Blood: +/- T 1 2 3 Alb:Cr Ratio _____ Cr _____ Alb _____	/ Height _____ Weight _____	Cr _____ Alb _____	☐ Urine ☐ Blood ☐ Saliva Episode (lab) _____	☐ Rash ☐ Abdominal pain ☐ Joint involvement ☐ Renal involvement ☐ Other ☐ Any medications (incl OTC) in the last 48 hours, list below:	

**list any persisting/recurring symptoms, other abnormal tests, progress on medication, concerns, etc.*

Outcome Page number ____

Histology & imaging *Please list date and results*			
☐ Renal biopsy	☐ Skin biopsy	☐ GI investigations	Other

Treatment/ Hospitalization Yes ☐ No ☐			
Date Admitted/ Date Commenced Treatment	Reason for admission	Treatment Medications commenced Indication/Dose/ Frequency	Discharge Date/ Med Stop Date

At 6 months	Date: ___/___/_____
☐ Discharged	☐ Remained under follow up: move onto extensive CRF Reason: _____

☐ All pages of CRF complete

8.3. **Appendix 3**

Full definitions used for recurrent / relapsing IgAV identified by the systematic literature review conducted in chapter 2.

Author. Y*ear*	**Definition used**	**Asymptomatic interval**
Garcia-Porrua, C. *et al. 2002 (306)*	Relapse if a patient diagnosed with HSP and asymptomatic for at least 1 month, presented a new flare of skin lesions or other systemic complication.	4 weeks
Gonzalez-Gay, M. A. *et al. 2004 (307)*	Relapse was considered if a child diagnosed with HSP and asymptomatic for at least 1 month presented a new flare of skin lesions or other systemic complications.	4 weeks
Trapani, S. *et al. 2005 (93)*	A relapse was defined as a new flare-up of skin lesions or other systemic complications following resolution of disease for at least 2 weeks.	2 weeks
Rigante, D. *et al. 2005 (97)*	With the term "relapse," we indicated disease resumption after a period of complete well-being for at least 1 month.	4 weeks
Shin, J. I. *et al. 2006 (8)*	Relapse was considered when a patient who was previously diagnosed as having HSP and who had been asymptomatic for at least 1 month presented with a new flare of cutaneous lesions or other systemic complications.	4 weeks
Cakir, M. *et al. 2006 (308)*	Recurrence was defined as a patient diagnosed with HSP who had been asymptomatic for at least 1 month presenting with a new episode of skin lesions or other systemic complications.	4 weeks
Alfredo, C. S. *et al. 2007 (96)*	Recurrence was defined as the presence of a fresh episode after a period of at least 3 months without symptoms.	3 months
Prais, D. *et al. 2007 (90)*	The reappearance of the characteristic purpuric rash (with or without associated symptoms), following a previous complete remission.	NA
Fretzayas, A. *et al. 2008 (91)*	Recurrence was considered when skin rash or other clinical signs and symptoms and/or laboratory findings relapsed after at least a 2-week period of well-being without cutaneous lesions or other systemic complications.	2 weeks
Anil, M. *et al. 2009 (185)*	A relapse was defined as a new flare-up of skin lesions or other systemic complications following resolution of the disease for at least two weeks.	2 weeks

Jauhola, O. *et al. 2010 (9)*	A recurrence was defined as an instance of a patient who had been asymptomatic for 1 month presenting with a new flare-up of skin lesions or other symptoms related to HSP.	4 weeks
Jauhola, O. *et al. 2010 (309)*	A recurrence of HSP disease was considered when a patient who had been asymptomatic for at least 1 month presented with a new flare-up of skin lesions or other symptoms related to HSP. .	4 weeks
Shin, J. I. *et al. 2011 (310)*	Relapses were considered to be present if a child, who has HSP diagnosis and has remained asymptomatic for at least 1 month, develops new skin lesions or other systemic complications.	4 weeks
Lardhi, A. A. *et al. 2012 (311)*	Recurrence was defined as a new flare-up of skin rash or other systemic complications following resolution of the disease for at least one month.	4 weeks
Teng, M. C. *et al. 2012 (141)*	A relapse was defined as the recurrence of clinical signs/symptoms or the occurrence of new symptoms after an initial remission, requiring the resumption of immunosuppressive therapy or an increased dose, within 3 months of remission.	3 months
Yilmaz, A. *et al. 2014 (312)*	Recurrence was defined as new skin rashes developed at least 4 weeks after disease recovery or reoccurrence of other signs.	4 weeks
Lee, Y. *et al. 2016 (313)*	Recurrence of HSP was defined as the recurrence of symptoms more than 1 month after remission.	4 weeks
Ma, D. Q. *et al. 2017 (314)*	HSP recurrence: HSP characteristic manifestations occurred again after at least 1 month since the symptoms of children diagnosed with HSP faded away;	4 weeks
Lei, W. T. *et al. 2018 (14)*	Those who received a second HSP diagnosis 3 months apart from the first HSP diagnosis were defined as having recurrent HSP (study subjects), which means a 3-months diagnosis-free interval between the first and second HSP diagnoses was required in order to define a recurrent HSP.	3 months
Xu, Y. *et al. 2019 (315)*	Relapse/recurrence was defined when a patient previously diagnosed with HSP and asymptomatic for at least 2 weeks, presented again a new flare of cutaneous lesions or other systemic manifestations of the vasculitis.	4 weeks
Rhim, J. W. *et al. 2019 (316)*	Recurrent cases of HSP, occurring ≥ 6 months after the first episode, were included.	6 months
Ekinci, R. M. K. *et al. 2019 (317)*	A relapse was defined as a new flare of cutaneous lesions or other manifestations in a patient at least four asymptomatic weeks after the initial HSP episode.	4 weeks

Karadag S, G. *et al*. *2019 (318)*	Relapse was described as presence of a new disease-related symptom after an asymptomatic period of at least 3 months.	3 months
Schinzel V. *et al*. *2019 (143)*	We defined recurrence when a new episode of purpura occurs after three months without any clinical signs or symptoms of the disease.	3 months
Wang, J. J. *et al. 2020 (319)*	Relapse/recurrence was defined when a patient previously diagnosed with HSP and asymptomatic for at least 2 weeks, presented again a new flare of cutaneous lesions or other systemic manifestations of the vasculitis.	2 weeks
Liao, C. H. M. *et al*. *2020 (12)*	Recurrence was defined as disease flare-up after complete remission and discontinuation of all medications for at least 3 months.	3 months
Ekinci, R. M. K. *et al*. *2020 (92)*	The presence of relapse was concluded when another episode of cutaneous lesions or other system involvements reappeared in a patient, at least one event-free month after the first manifestations.	4 weeks
Gokce, S *et al*. *2020 (139)*	The recurrence was defined when a patient previously diagnosed with HSP and was asymptomatic for at least 1 month without medication, presented again with a new flare of cutaneous lesions or other systemic manifestations of the vasculitis.	4 weeks
Fan, G. Z. *et al. 2020 (140)*	Relapse/recurrence was defined when a patient previously diagnosed with HSP and asymptomatic for at least 2 weeks after treatment, presented again a new flare of cutaneous lesions or other systemic manifestations of the vasculitis.	2 weeks
Zhang, Y. *et al*. *2021 (320)*	Recurrence of HSP, which was defined as patients with a pre-existing diagnosis of HSP but having no symptoms for at least 4 weeks who presented symptoms again.	4 weeks
Sestan, M. *et al*. *2021 (321)*	Relapse was defined as a new flare of clinical signs attributable to IgAV in a patient previously diagnosed with IgAV after an asymptomatic period of at least 1 month.	4 weeks
Rubino, C. *et al*. *2021 (69)*	A new flare of cutaneous lesions and/or other IgAV features, in a patient previously diagnosed with IgAV and asymptomatic for at least 1 month, was defined as relapse.	4 weeks
Sestan, M. *et al*. *2022 (130)*	Relapse was defined as a new flare of clinical signs attributable to IgAV in a patient previously diagnosed with IgAV after an asymptomatic period of at least 1 month.	4 weeks
Farisogullari, B. *et al. 2022 (142)*	Relapse: requiring the resumption of corticosteroids or other immunosuppressive therapies, or as a significant increase in glucocorticoid dose (at least 50%).	NA

8.4. **Appendix 4**

Proteins assessed in chapter 4 by R&Ds Systems Human Kidney Biomarker Array Kit ('Kit K') and Human XL Cytokine Array Kit ('Kit C').

Name used	Full name	Alternative names	Assessed by	
			Kit K	Kit C
Adiponectin	-	Arcp30	x	x
Aminopeptidase N	-	ANPEP	x	
Angiogenin	-	-		x
Angiopoietin-1	-	Ang1, ANGPT1		x
Angiopoietin-2	-	Ang-2, ANGPT2		x
AGT	Angiotensinogen	Serpin A8	x	
Annexin V	-	-	x	
ApoA1	Alipoprotein A	-		x
BAFF	B-cell activating factor	BLyS, TNFSF13B		x
BDNF	Brain-derived neurotrophic factor	Abrineurin		x
β2-Microglobulin	-	β2M	x	
C5/C5a	Complement component C5/C5a	-		x
CD14	Cluster of differentiation 14	-		x
CD30	Cluster of differentiation 30	TNFRSF8		x
CD31	Cluster of differentiation 31	PECAM-1		x
CD40 Ligand	Cluster of differentiation 40 ligand	CD154 TNFSF5		x
Chitinase-3-like-1	-	YKL-40		x
Clusterin	-	Apolipoprotein J	x	
CFD	Complement factor D	Adipsin		x
Cripto-1	-	TDGF-1		x
CRP	C-reactive protein	-		x
CXCL16	Chemokine (C-X-C motif) ligand 16	-	x	
Cyr61	Cysteine-rich angiogenic inducer 61	CCN1	x	
Cystatin C	-	CST3, ARMD11	x	x
Dkk-1	Dickkopf-related protein 1	SK, dickkopf WNT signalling pathway inhibitor 1		x
DPPIV	Dipeptidyl peptidase-4	CD26, DPP4, Dipeptidyl-peptidase IV	x	x
EGF	Epidermal growth factor	-	x	x

EGF-R	Epidermal growth factor receptor	ErbB1, HER1	x	
EMMPRIN	Extracellular matrix metalloproteinase inducer	Basigin, CD147		x
ENA-78	Epithelial-derived neutrophil-activating peptide 78	CXCL5		x
Endoglin		CD105, ENG		x
L-FABP	liver-type fatty acid-binding protein	FABP1	x	
Fas Ligand		CD95L, CD178, TNFSF6		x
Fetuin A		AHSG	x	
FGF-7	Fibroblast growth factor 7	KGF		x
FGF-19	Fibroblast growth factor 19	-		x
FGF-basic	Fibroblast growth-factor	FGF-2		x
Flt-3 Ligand	Fms-related tyrosine kinase 3 ligand	FLT3LG		x
G-CSF	Granulocyte colony-stimulating factor	CSF3		x
GDF-15	Growth/differentiation factor 15	MIC-1		x
GH	Growth hormone	Somatotropin		x
GM-CSF	Granulocyte macrophage colony-stimulating factor	CSF2		x
CXCL1	Chemokine (C-X-C motif) ligand 1 (CXCL1)	GROα, MSGA-α	x	x
HGF	Hepatocyte growth factor	Scatter factor, SF		x
ICAM-1	Intercellular Adhesion Molecule 1	CD54		x
IFN-γ	Interferon gamma	IFNG		x
IGFBP-2	Insulin-like growth factor-binding protein 2	-		x
IGFBP-3	Insulin-like growth factor-binding protein 3	-		x
IL-10	Interleukin-10	-	x	x
IL-11	Interleukin-11	-		x
IL-12p70	Interleukin-12p70	-		x
IL-13	Interleukin-13	-		x
IL-15	Interleukin-15	-		x
IL-16	Interleukin-16	-		x
IL-17A	Interleukin-17A	-		x

IL-18 BP	Interleukin-18 binding protein	IL-17, CTLA8		x
IL-19	Interleukin-19	-		x
IL-1α	Interleukin-1 alpha	IL-F1		x
IL-1β	Interleukin-1 beta	IL-F2		x
IL-1RA	Interleukin-1 receptor antagonist	IL-F3	x	x
IL-2	Interleukin-2	-		x
IL-22	Interleukin-22	IL-TIF		x
IL-23	Interleukin-23	IL-23A, SGRF		x
IL-24	Interleukin-24	C49A, FISP, MDA-7, MOB-5, ST16		x
IL-27	Interleukin-27	-		x
IL-3	Interleukin-3	-		x
IL-31	Interleukin-31	-		x
IL-32	Interleukin-32	-		x
IL-33	Interleukin-33	C9orf26, DVS27, NF-HEV		x
IL-34	Interleukin-34	C16orf77		x
IL-4	Interleukin-4	-		x
IL-5	Interleukin-5	-		x
IL-6	Interleukin-6	-	x	x
IL-8	Interleukin-8	CXCL8		x
IP-10	Interferon gamma-induced protein 10	CXCL10		x
I-TAC	Interferon-inducible T-cell alpha chemoattractant	CXCL11, SYCB9B		x
Leptin	-	OB		x
LIF	Leukemia inhibitory factor	-		x
NGAL	Neutrophil gelatinase-associated lipocalin	Lipocalin-2, LCN2, Siderocalin	x	x
MCP-1	Monocyte chemoattractant protein-1	CCL2, MCAF	x	x
MCP-3	Monocyte chemoattractant protein 3	CCL7, MARC		x
M-CSF	Macrophage colony-stimulating factor	CSF1		x
MIF	Macrophage migration inhibitory factor	-		x

MIG	Monokine induced by gamma interferon	CXCL9		x
MIP-1α/MIP-1β	Macrophage inflammatory proteins 1 alpha /1 beta	CCL3/CCL4		x
MIP-3α	Macrophage inflammatory protein 3 alpha	CCL20, Exodus-1, LARC		x
MIP-3β	Macrophage inflammatory protein 3 beta	CCL19, ELC		x
MMP-9	Matrix metallopeptidase 9	CLG4B, Gelatinase B	x	x
Myeloperoxidase	-	MPO, Lactoperoxidase		x
Neprilysin	-	CD10	x	
Osteopontin	-	OPN		x
PDGF-AA	Platelet-derived growth factor AA	-		x
PDGF-AA/BB	Platelet-derived growth factor AA/BB	-		x
Pentraxin 3	-	PTX3, TSG-14		x
PF4	Platelet factor 4	CXCL4		x
PSA	Prostate-specific antigen	KLK-3	x	x
RAGE	Receptor for advanced glycation endproducts	AGER	x	x
RANTES	Regulated on activation, normal T cell expressed and secreted	CCL5		x
RBP-4	Retinol Binding Protein 4	-	x	x
Relaxin-2	-	RLN2, RLXH2		x
Renin	-	Angiotensinogenase	x	
Resistin	-	ADSF, FIZZ3, RETN	x	x
SCF	Stem cell factor	-	x	
SDF-1α	Stromal cell-derived factor 1 alpha	CXCL12, PBSF		x
Serpin A3	-	α1-antichymotrypsin	x	
Serpin E1	-	PAI-I, PAI-1, Nexin		x
SHBG	Sex hormone binding globulin	ABP		x
ST2	Suppression of tumorigenicity 2	IL-1 R4, IL1RL1, ST2L		x
TARC	Thymus- and activation-regulation chemokine	CCL17		x
TfR	Transferrin	CD71, TFR1, TFRC, TRFR		x
TGF-α	Transforming growth factor alpha	TGFA		x
Thrombospondin-1	-	THBS1, TSP-1	x	x

KIM-1	Kidney injury molecule 1	TIM-1, HAVCR	x	
TIM-3	T-cell immunoglobulin and mucin-domain containing-3	HAVCR2		x
TNF R1	Tumour necrosis factor receptor 1	TNFRSF1A	x	
TNF-α	Tumour necrosis factor alpha	TNFSF1A	x	x
TFF3	Trefoil Factor 3	ITF, TFI	x	x
TWEAK	Tumor necrosis factor-like weak inducer of apoptosis	TNFSF12	x	
uPA	Urokinase-type plasminogen activator	Urokinase	x	
uPAR	Urokinase plasminogen activator receptor	PLAUR		x
VCAM-1	Vascular cell adhesion molecule 1	CD106	x	x
VEGF	Vascular endothelial growth factor	VPF		x
VEGF-A	Vascular endothelial growth factor-A	VPF-A	x	
Vitamin D BP	Vitamin D Binding Protein	VDB, DBP, VDBP		x

8.5. **Appendix 5**

Full results from chapter 4: Human Kidney Biomarker Array Kit.

Protein	IgAVN vs IgAVwoN		IgAVN vs HC		IgAVwoN vs HC	
	Fold-change	***p*-value**	**Fold-change**	***p*-value**	**Fold-change**	***p*-value**
Adiponectin	2.9919	0.57799	4.3938	0.27887	1.4686	0.32622
Angiotensinogen	6.4865	0.0096	22.603	0.007151	3.4846	0.054706
Annexin V	3.1693	0.45212	4.5046	0.37053	1.4213	0.90716
ANPEP	1.6726	0.93273	2.8759	0.47447	1.7194	0.2887
β2 microglobulin	1.1466	0.93184	3.1066	0.24131	2.7093	0.18944
Clusterin	1.7263	0.73026	3.8383	0.85582	2.2235	0.10659
CXCL16	3.728	0.01332	4.4656	0.013257	1.1979	0.58787
Cyr61	1.9483	0.5891	3.9752	0.41352	2.0403	0.83646
Cystacin-C	1.1894	0.97656	2.8185	0.29247	2.3698	0.17507
DPPIV	1.7591	0.61695	3.0626	0.19653	1.741	0.15967
EGF	1.0829	0.93131	3.0313	0.22998	2.7992	0.20843
EGF-R	5.8432	0.00232	7.8429	0.000424	1.3422	0.56969
FABP1	2.1775	0.44138	5.8558	0.13951	2.6892	0.42364
Fetuin-A	1.0103	0.62367	2.738	0.85618	2.71	0.1657
CXCL1	1.7859	0.53345	4.9731	0.20574	2.7846	0.53907
IL-10	1.5736	0.65926	4.8126	0.34642	3.0583	0.64184
IL-1RA	5.6892	0.12162	4.8312	0.095243	0.84918	0.51867
IL-6	2.5843	0.19587	5.5279	0.036444	2.139	0.49628
KIM-1	4.0891	0.00382	4.1496	0.00115	1.0148	0.93212
NGAL	1.1769	0.34533	1.8393	0.16901	1.5629	0.71228
MCP-1	3.3959	0.04690	11.956	0.001066	3.5206	0.001091
MMP-9	0.5616	0.93974	2.6748	0.42349	4.7628	0.45181
Neprilysin	1.7023	0.48842	2.3959	0.19983	1.4075	0.322
PSA	1.2936	0.7259	3.9295	0.45469	3.0377	0.71787
RAGE	0.82103	0.85485	1.689	0.50084	2.0571	0.40355
RBP4	1.0095	0.82887	2.2656	0.32605	2.2444	0.12205
Renin	1.2747	0.71454	3.7978	0.40608	2.9795	0.68952
Resistin	0.95591	0.9099	1.1464	0.40525	1.1993	0.44064
SCF	1.8794	0.3667	5.3492	0.19574	2.8463	0.98275
Serpin_A3	1.8348	0.9453	7.0708	0.40647	3.8537	0.2036
TFF3	1.1309	0.93257	2.8717	0.25503	2.5394	0.21273
Thrombospondin-1	1.383	0.71873	3.6518	0.39685	2.6405	0.67322
TNF-R1	0.9231	0.74193	1.7886	0.36378	1.9376	0.17036
TNF-α	1.6373	0.44583	2.6742	0.36577	1.6333	0.96245
TWEAK	2.4252	0.5586	4.1908	0.49631	1.728	0.96588
uPA	1.2215	0.88152	3.0317	0.20082	2.482	0.15934
VCAM-1	2.5729	0.22071	4.0982	0.15574	1.5928	0.3686
VEGF	1.2105	0.40523	1.5217	0.14103	1.257	0.34796

8.6. **Appendix 6**

Full results from chapter 4: Human XL Cytokine Array Kit.

Protein	IgAVN vs IgAVwoN		IgAVN vs HCs		IgAVwoN vs HCs	
	Fold-change	***p*-value**	**Fold-change**	***p*-value**	**Fold-change**	***p*-value**
Adiponectin	1.8287	0.57097	3.2492	0.17803	1.7768	0.21624
Angiogenin	1.6477	0.33875	2.212	0.11892	1.3425	0.13731
Angiopoietin-1	1.4268	0.32607	3.4687	0.033806	2.4312	0.35446
Angiopoietin-2	1.1577	0.7162	3.2967	0.05545	2.8476	0.12544
Apolipoprotein A 1	6.0461	0.020463	5.9723	0.034772	0.98779	0.58263
BAFF	9.7227	0.026053	26.468	0.003899	2.7223	0.078796
BDNF	1.0461	0.75692	2.6174	0.019991	2.502	0.063713
C5/C5a	4.5968	0.032956	6.5258	0.011829	1.4196	0.63215
CD14	2.0131	0.3988	2.9582	0.1626	1.4695	0.28197
CD30	0.52959	0.12288	0.75429	0.49646	1.4243	0.23274
CD31	0.86691	0.62807	1.7843	0.10109	2.0583	0.062136
CD40_Ligand	3.0402	0.044625	7.1976	0.007591	2.3675	0.070268
Chitinase 3-like 1	0.86917	0.75871	2.4447	0.10388	2.8127	0.37182
CFD	2.6001	0.008623	3.4104	0.004554	1.3116	0.40523
Cripto-1	7.8132	0.014841	7.1403	0.026301	0.91387	0.86666
CRP	2.4108	0.2663	3.0548	0.18704	1.2671	0.89643
Cystatin-C	1.2067	0.29366	1.1915	0.40639	0.98741	0.82491
Dkk-1	1.1093	0.56197	2.5345	0.011049	2.2847	0.1296
DPPIV	3.1747	0.1454	4.0274	0.075809	1.2686	0.4476
EGF	1.0446	0.9617	2.728	0.26843	2.6115	0.23998
EMMPRIN	1.0361	0.99501	2.001	0.25474	1.9313	0.21085
ENA-78	3.7452	0.058576	7.4035	0.015579	1.9768	0.11815
Endoglin	1.7528	0.020648	2.7075	0.014267	1.5447	0.19651
Fas Ligand	2.0645	0.086281	3.757	0.007516	1.8198	0.29808
FGF-19	1.5387	0.42579	3.2109	0.070494	2.0868	0.21824
FGF-7	1.4476	0.44304	2.9148	0.067729	2.0136	0.27496
FGF-basic	1.5811	0.29393	2.9534	0.068045	1.8679	0.50599
Flt-3 ligand	1.6921	0.13051	2.7008	0.018979	1.5961	0.22565
G-CSF	1.5053	0.21954	3.577	0.003326	2.3763	0.18161
GDF-15	1.5685	0.71341	2.3347	0.28713	1.4885	0.20007
GH	2.5913	0.067259	6.2705	0.003865	2.4198	0.017402
GM-CSF	1.4378	0.23444	3.2279	0.016377	2.245	0.3229
CXCL1	2.0059	0.022027	4.2743	0.000116	2.1309	0.016553
HGF	1.8013	0.02783	4.2975	0.00116	2.3858	0.019725
ICAM-1	4.0385	0.011899	10.249	6.39E-05	2.5377	0.14455
IFN-γ	1.2014	0.60944	3.2454	0.030262	2.7014	0.13751
IGFBP-2	1.7674	0.20262	4.8827	0.010683	2.7627	0.30689
IGFBP-3	3.4145	0.022753	10.73	2.13E-05	3.1426	0.11542
IL-10	1.6549	0.28815	3.3107	0.032085	2.0006	0.20312

IL-11	1.5168	0.44203	2.8766	0.098683	1.8965	0.30443
IL-12p70	1.0739	0.81759	3.0354	0.067595	2.8266	0.1222
IL-13	1.2101	0.60282	3.4298	0.016215	2.8343	0.049089
IL-15	1.4094	0.28861	3.153	0.01116	2.2371	0.18352
IL-16	1.2732	0.37424	2.7409	0.016	2.1528	0.27178
IL-17a	1.2603	0.87964	2.0202	0.31992	1.603	0.27878
IL-18 BP	1.0733	0.97381	2.6403	0.27038	2.4599	0.21658
IL-19	1.5401	0.31964	3.8165	0.008649	2.4781	0.029131
IL-1α	0.86362	0.87519	2.5854	0.10271	2.9936	0.084115
IL-1β	1.0638	0.72184	2.5271	0.0826	2.3755	0.21056
IL-1RA	1.2221	0.97565	2.3637	0.23858	1.9341	0.10512
IL-2	1.215	0.56018	2.6339	0.11002	2.1679	0.35638
IL-22	1.834	0.24804	4.4014	0.019852	2.3998	0.052279
IL-23	1.3567	0.4313	3.2976	0.02441	2.4307	0.16244
IL-24	1.2106	0.50425	3.1891	0.018619	2.6344	0.14649
IL-27	1.133	0.57041	3.2683	0.022913	2.8847	0.15347
IL-3	1.1007	0.57302	3.8068	0.074403	3.4585	0.32704
IL-31	1.3794	0.37148	3.2694	0.015051	2.3702	0.14672
IL-32	1.0852	0.55789	3.5242	0.004058	3.2475	0.079091
IL-33	1.3956	0.36308	3.7335	0.006638	2.6752	0.11432
IL-34	1.1551	0.55351	2.5888	0.025418	2.2413	0.20391
IL-4	1.989	0.077582	4.6257	0.003759	2.3257	0.035862
IL-5	2.029	0.046712	4.611	0.00292	2.2726	0.015636
IL-6	1.7949	0.29276	3.2726	0.051585	1.8233	0.19671
IL-8	1.9038	0.11659	2.8859	0.038825	1.5159	0.80768
IP-10	1.7876	0.12758	2.8273	0.022831	1.5816	0.12661
I-TAC	1.2475	0.37007	2.8928	0.008766	2.319	0.2042
PSA	1.0379	0.88677	2.5245	0.068415	2.4323	0.090241
Leptin	1.6779	0.2682	4.3158	0.00938	2.5722	0.022296
LIF	2.4099	0.00752	5.5375	0.001362	2.2978	0.06466
NGAL	1.0664	0.4986	2.063	0.10666	1.9345	0.77922
MCP-1	1.8851	0.06115	4.1117	0.00577	2.1812	0.13351
MCP-3	1.3311	0.37702	3.2094	0.016332	2.4111	0.17594
M-CSF	1.1701	0.28417	1.7828	0.08188	1.5236	0.1689
MIF	2.1612	0.015038	3.1333	0.006118	1.4498	0.29744
MIG	1.701	0.10966	3.8285	0.000975	2.2508	0.2024
MIP-1α/MIP-1β	1.4109	0.27706	2.5586	0.013899	1.8134	0.31822
MIP-3α	1.3186	0.34885	2.5306	0.017659	1.9192	0.2996
MIP-3β	0.92472	0.48424	3.488	0.012799	3.7719	0.32589
MMP-9	0.7438	0.72013	1.6703	0.087044	2.2456	0.49677
Myeloperoxidase	0.42741	0.3448	3.7338	0.059293	8.7358	0.88862
Osteopontin	1.2659	0.84319	2.1902	0.53528	1.7301	0.19047
PDGF-AA	0.95234	0.73517	2.2445	0.027002	2.3568	0.11891
PDGF-AA/BB	1.4861	0.23584	4.2025	0.007355	2.8278	0.13029

Pentraxin-3	1.1424	0.73195	3.0177	0.035324	2.6416	0.072243
PF4	2.8905	0.26081	7.528	0.035516	2.6044	0.16161
RAGE	0.61426	0.39621	1.2002	0.66954	1.954	0.27215
RANTES	1.9093	0.12788	4.4314	0.010905	2.321	0.1824
RBP-4	1.3193	0.70566	2.249	0.17302	1.7047	0.18646
Relaxin-2	1.2276	0.51042	2.909	0.048065	2.3697	0.26231
Resistin	1.5577	0.31074	1.4971	0.3415	0.96111	0.83688
SDF-1a	1.2941	0.2665	1.9324	0.070332	1.4933	0.23185
Serpin E1	2.491	0.033753	8.1136	9.91E-05	3.2572	0.069064
SHBG	7.5808	0.007742	13.339	0.00358	1.7595	0.24373
ST2	3.3299	0.014425	8.1713	0.000415	2.4539	0.082604
TARC	1.4684	0.33971	4.1432	0.010824	2.8215	0.073098
TFF3	1.0261	0.99463	2.7465	0.26291	2.6768	0.20413
TfR	1.9306	0.086845	5.6231	0.000308	2.9126	0.05055
TGF-α	0.8594	0.92868	2.2638	0.027643	2.6341	0.15107
Thrombospondin-1	0.91736	0.78108	2.6222	0.029302	2.8584	0.153
TIM-3	1.117	0.99997	2.6252	0.27146	2.3503	0.18122
TNF-α	1.1218	0.46899	2.3231	0.023407	2.0709	0.3851
uPAR	0.76021	0.44874	1.3151	0.33489	1.7299	0.15929
VCAM-1	2.3545	0.2753	4.2414	0.096912	1.8014	0.1735
VEGF	1.0948	0.79309	1.5399	0.22317	1.4066	0.28407
Vitamin D BP	2.5958	0.33743	4.3044	0.10077	1.6582	0.10032