UNIVERSITY OF LIVERPOOL

Methods of measuring disease activity in paediatric IgA vasculitis

Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Master of Philosophy

By Chloe Williams

[July 2021]

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.

Chloe Williams

[July 2021]

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Abstract

Introduction: IgA vasculitis (IgAV, Henoch-Schönlein purpura, HSP) is the most common vasculitis of childhood and currently contributes to 1-2% of all chronic kidney disease (CKD) stage 5. New methods of measuring disease activity are required to improve the standard of care given. The aim of this thesis is to evaluate methods of measuring disease activity in IgAV using urine biomarkers and a disease-specific scoring tool.

Methods: Firstly, a systematic literature review was performed using 4 search engines and a search term strategy with predefined inclusion and exclusion criteria. Promising biomarkers were divided in terms of traditional or novel and described using statistical significance and area under the curve (AUC) values. Secondly, a specific disease activity scoring tool (the IgA-VAS) was developed and preliminarily validated in a cohort of paediatric patients with IgAV. Test validity, concurrent validity and inter-rater agreement were assessed retrospectively. A randomly selected subgroup were also scored using a visual analogue scale.

Results: The systematic review identified 13 eligible studies. A total of 2,446 paediatric patients were included: healthy controls (n=761), children with IgAV-N (n=1,236) and children with IgAV without nephritis (IgAV-noN, n=449). 51% were male, median age 7.9 years. The traditional markers, 24-hour protein quantity and urine protein:creatinine ratio were deemed acceptable for assessing severity of nephritis (AUC <0.8). Urinary albumin concentration (Malb) performed well (AUC 0.81-0.98). The most promising novel urinary biomarkers in predicting presence of nephritis were kidney injury molecule-1 (KIM-1) (AUC 0.93), monocyte chemotactic protein-1 (MCP-1) (AUC 0.83), N-acetyl-β-glucosaminidase (NAG) (0.76-0.96), and angiotensinogen (AGT) (AUC not available). Urinary KIM-1, MCP-1, and NAG appeared to correlate with disease severity. The IgA-VAS consists of 40 manifestations, each with a score from 0-10, divided into 5 domains: cutaneous, gastrointestinal, musculoskeletal, renal and other. For preliminary validation, retrospective scoring was performed in a single tertiary centre over a 5-year period. 153 children met the inclusion criteria: 54% were male with a median age of 5.7 years (range 0.6-16.7). Median total scores for the IgA-VAS were 7/125 (range 2-31) and 5/125 (range 2-29) for rater 1 and rater 2 respectively. Median PVAS scores were 6/63 (range 2-25) and 5/63 (range 2-20). Correlation between all overlapping domains of the two tools was strong (all $r>0.5$, $p<0.001$). Inter-rater reliability overall was low for both tools (0.131 and 0.225, $p<0.001$). For the IgA-VAS, inter-rater reliability was low for the cutaneous, renal, and other domains (0.332, 0.237, 0.288 $p<0.001$) and high for the gastrointestinal and musculoskeletal domains (0.543 and 0.667, $p<0.001$). The general, cutaneous, and renal subsystems in the PVAS had a low inter-rater reliability (0.347, 0.213, 0.304, $p<0.001$) and was better for the abdominal domain (0.579, $p<0.001$). The IgA-VAS moderately

correlated with the visual analogue scale for both raters (r=0.482, r=0.362, p<0.05), however the PVAS strongly correlated with rater 1 (r=0.504, p=0.004) and moderately correlated with rater 2 (r=0.372, p=0.043).

Conclusion: Future studies should focus on multicentre prospective studies for biomarker discovery and validation of the IgA-VAS in a large cohort of paediatric patients.

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I would like to thank FAIR, who made this year possible through their generous financial support. I hope that this work and the work of my colleagues will continue to improve the lives of those living with autoimmune conditions.

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Outputs arising from this thesis

Publications

Williams, C.E.C., Toner, A., Wright, R.D. *et al.* A systematic review of urine biomarkers in children with IgA vasculitis nephritis. *Pediatr Nephrol* (2021). https://doi.org/10.1007/s00467-021-05107-7 (Appendix 1)

Presentations

Chloe E. C. Williams, Aileen Toner, Rachael D, Wright, *et al.* Poster presentation at the Royal College of Paediatrics and Child Health (RCPCH) Conference (June 2021). A systematic review of urine biomarkers in children with IgA vasculitis nephritis.

Chloe E. C. Williams, Aileen Toner, Rachael D, Wright, *et al*. Oral pitch presentation at the 53rd European Society of Paediatric Nephrology (ESPN) Annual Meeting (September 2021). A systematic review of urine biomarkers in children with IgA vasculitis nephritis.

Chloe E. C. Williams, Jared Murphy, Tom Dowsett, *et al*. Oral pitch presentation at the 53rd European Society of Paediatric Nephrology (ESPN) Annual Meeting (September 2021). The development and preliminary validation of a scoring tool for monitoring disease activity in patients with IgA vasculitis (HSP).

COVID-19 Disruptions

During the course of this year, the COVID-19 pandemic has disrupted and changed aspects of my MPhil degree. The original aim of this thesis was to discover urine biomarkers in children with IgA vasculitis nephritis, however due to periods of self-isolation and delays in both the opportunity to recruit patients and equipment delivery, the laboratory aspects were not conducted in time to be included in this thesis. Despite these unforeseen changes, I had the opportunity to learn laboratory skills by running practice biomarker assays and processing the urine and serum samples of the healthy controls which I helped recruit to the IgA Vasculitis Study. I hope to complete the biomarker assay on a small sample of patients after the submission of this thesis, subject to arrival of the equipment, which will ultimately contribute to further work done at Alder Hey.

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List of Abbreviations

α_1-MG	Alpha 1 microglobulin
β2-MG	Beta 2-microglobulin
24h-UPRO	24-hour urinary protein
ACE-i	Angiotensin converting enzyme inhibitor
ACR	Albumin:creatinine ratio
AGT	Angiotensinogen
AKI	Acute kidney injury
ANCA	Anti-neutrophil cytoplasm antibodies
ARB	Angiotensin receptor blocker
AUC	Area under the curve
BP	Blood pressure
BVAS	Birmingham Vasculitis Activity Score
CKD	Chronic kidney disease
CRP	C-reactive protein
DAS-28	Disease Activity Score-28 for Rheumatoid Arthritis
EDRN	Early Detection Research Network
eGFR	Estimated glomerular filtration rate
ENT	Ear, nose, and throat
ESR	Erythrocyte sedimentation rate
ESRF	End stage renal failure
EULAR	European League Against Rheumatology
FSP-1	Fibroblast-specific protein
GI	Gastrointestinal
HSP	Henoch-Schönlein purpura
Ig	Immunoglobulin
IgAV	IgA vasculitis
IgAV-N	IgA vasculitis with nephritis
IgAV-noN	IgA vasculitis without nephritis
IgG/Cr	Immunoglobulin G/creatinine ratio
IgAN	IgA nephropathy
IL	Interleukin

ISKDC	International Study of Kidney Disease in Children
ITGB1	Integrin beta-1
IV	Intravenous
KIM-1	Kidney injury molecule-1
KDIGO	Kidney Disease Improving Global Outcomes
L-FABP	Liver-fatty acid binding protein
Malb	Microalbumin
MCP-1	Monocyte chemoattractant protein-1
MEST-C	Mesangial and endocapillary hypercellularity, segmental sclerosis, interstitial fibrosis/tubular atrophy, and the presence of crescents
MIF	Macrophage migration inhibitory factor
MMP-9	Matrix metalloproteinase-9
NAG	N-acetyl-β-glucosaminidase
NGAL	Neutrophil gelatinase-associated lipocalin
NSAID	Non-steroidal anti-inflammatory drug
PCR	Protein:creatinine ratio
PGA	Physician global assessment
PRINTO	Paediatric Rheumatology International Trials Organisation
PReS	Paediatric Rheumatology European Association
PsARC	Psoriatic Arthritis Response Criteria
PVAS	Paediatric Vasculitis Activity Score
RF	Rheumatoid factor
ROC	Receiver operating characteristic
RRT	Renal replacement therapy
SHARE	Single Hub and Access point for paediatric Rheumatology in Europe
SLE	Systemic lupus erythematosus
SLEDAI-2K	Systemic Lupus Erythematosus Disease Activity Index 2000
SQC	Semi-quantitative classification
TfR	Transferrin
TIMP-1	Tissue inhibitor matrix metalloproteinase-1
UAGT	Urinary angiotensinogen
UK	United Kingdom
U-PCR	Urinary protein:creatinine ratio

1. Introduction

1.1 Immunoglobulin A vasculitis

Immunoglobulin A vasculitis (IgA vasculitis, IgAV), formerly Henoch-Schönlein purpura (HSP) is a small vessel, hypersensitivity vasculitis that predominates in childhood. It often presents acutely with clinical features which can include a palpable purpuric rash, gastrointestinal symptoms, arthralgia/arthritis, and renal involvement.

1.1.2 Epidemiology

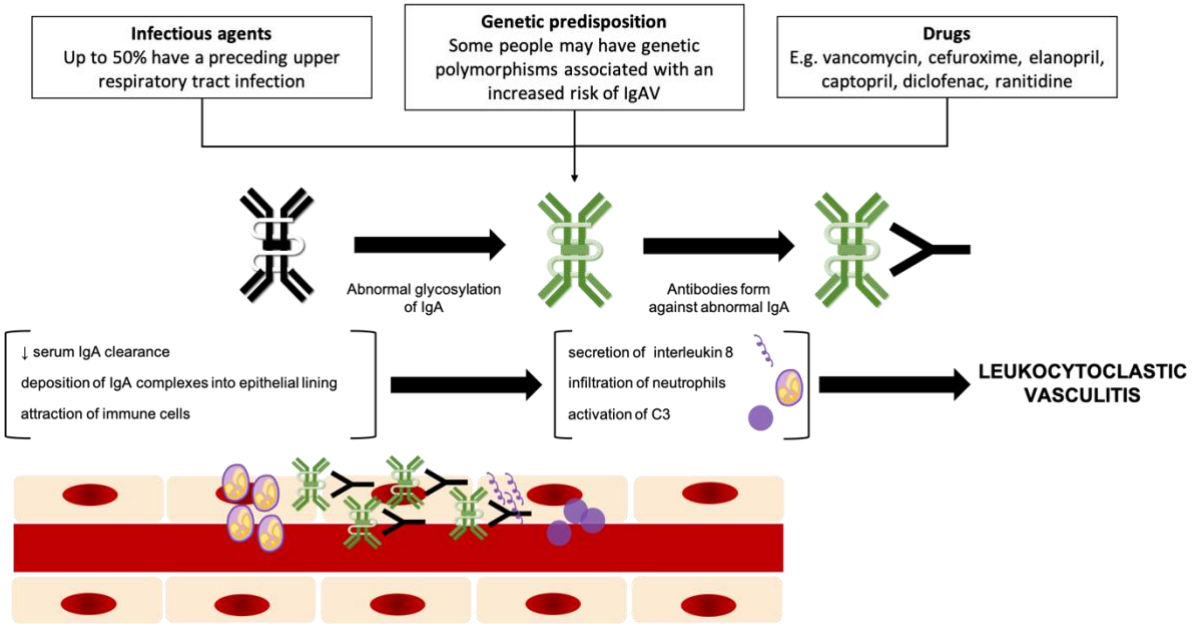
IgAV is a rare condition and is estimated to affect 3-27 per 100,000 children per year (2). 90% of childhood cases develop under the age of 10 years, with a peak prevalence in children aged 4-6 years (3). It is extremely rare in infants and uncommon in teenagers and adults, however these patient groups are more likely to experience a more complicated disease course. There is a slight male predominance, and the overall incidence decreases with age. Slight differences are seen in childhood-onset IgAV when compared to adult-onset, with abdominal pain less commonly seen as a presenting complaint in adults and adults are more likely to develop arthritis. There is a clear seasonal variation with IgAV with increased number of cases during winter, spring and autumn. This may be due to its association with preceding viral infections that are often seen in the days or weeks prior to presentation (4).

1.1.3 Pathophysiology

The exact pathophysiology of IgAV is still unknown, however due to the increased serum concentration of galactose deficient IgA1 levels in the serum, it is thought that aberrant IgA glycosylation is a contributor to the mechanism of disease (5). Immune complexes containing IgA1 in the serum cannot be cleared normally so deposit in the small vessels activating a humoral autoimmune response and subsequent inflammation (**Figure *1***) (6). In the skin, for example, this results in vasodilation and endothelial activation leading to extravasation of blood into the skin forming the typical rash.

There is believed to be a genetic element to IgAV, partly because of the galactose deficient IgA1 seen in the siblings of patients with clinical IgAV but also due to the ethnic variation of disease prevalence (7). A previous systematic review found the polymorphisms *HLA-DRB1*01, 07*, and *11* to be the most convincingly associated with an increased risk of IgAV (8). It is also thought that there may be genetic abnormalities resulting in a defected glycosylation pathway (7).

Figure 1 | The pathophysiology of IgA vasculitis.



1.1.4 Clinical features

1.1.4.1 Cutaneous

An erythematous, palpable purpuric rash is the most characteristic and common cutaneous manifestation of IgAV. It is usually symmetrical can be associated with petechiae and areas of bruising, occasionally developing into ulcers and bullae and rarely into necrotic or gangrenous regions. The rash predominantly starts on the lower limbs and buttocks, occasionally spreading to the arms, infrequently to the trunk, and rarely to the head and neck. The rash is self-resolving in the vast majority of cases.

1.1.4.2 Musculoskeletal

Involvement of the joints typically comes in the form of arthralgia and/or an oligoarthritis. Previous studies have suggested the rate of joint involvement to be 78.5-90% (9, 10). The most common joints affected appear to be the lower limb joints such as the feet or ankles (85%) followed by the knees (38%) (9). Joint involvement seldom has any long-term effects and supportive management is usually sufficient.

1.1.4.3 Gastrointestinal

Gastrointestinal (GI) symptoms can occur before the onset of the cutaneous symptoms in 5% of patients which may lead to incorrect clinical diagnosis until the rash manifests itself. GI manifestations usually come in the form of colicky abdominal pain due to bowel angina and in some cases involvement of the GI tract may be more serious, with GI bleeding, melaena or intussusception occurring (11). Some children may also experience associated nausea, vomiting and/or diarrhoea.

1.1.4.4 Renal

Renal involvement in IgAV (IgA nephritis, IgA-N) can range from microscopic haematuria to end-stage renal failure. It usually presents within the first 6 weeks but may develop later so monitoring is recommended for 6 months following diagnosis. At diagnosis, all patients should have a urinalysis performed to screen for renal involvement. Any patients with signs of worsening or persisting nephritis proceed to have a kidney biopsy performed. Criteria for this include: severe proteinuria (i.e. >250mg/mmol) for >4 weeks but may be considered sooner; persistent moderate proteinuria (100-150 mg/mmol for >4 weeks); and/or an impaired eGFR (<80 ml/min/1.73m^2) (12).

1.1.4.5 Other

Other manifestations of IgAV include orchiditis which is seen in 14% of male patients (9), more rarely, neurological involvement (headache, seizure) and pulmonary haemorrhage.

1.1.4 Diagnosis

According to the Single Hub and Access point for paediatric Rheumatology in Europe (SHARE) initiative, diagnosis of IgAV should be based on clinical features and it is distinguished from other forms of vasculitis using the 2008 EULAR/PRINTO/PRES classification criteria (**Table *1***) (13). This requires the presence of lower-limb predominant purpura, in the absence of thrombocytopaenia, with at least one of: abdominal pain, histopathology, arthritis or arthralgia, or renal involvement. A lower limb predominant purpuric rash and IgA deposition has both a sensitivity and specificity of >80%, whilst that of abdominal pain is >60%. Arthritis/arthralgia is more sensitive (78%) than it is specific, and proteinuria/haematuria is more specific (70%) than sensitive. Overall, the sensitivity and specificity of the EULAR/PRINTO/PRES criteria is high (100% and 87%) (13).

1.1.5 Histology

The International Study of Kidney Disease in Children (ISKDC) classification of histological findings on renal biopsies was published in 1977 and is used to histologically categorise features of IgAV-N (**Table *2***). Although histology provides a definitive picture of renal inflammation, it is an invasive procedure with recognised risks such as post-operative bleeding. More recently, efforts have been made to improve the accuracy of the histological reporting. This had led to the possibility of using descriptions in addition to the ISKDC classification, such as a modified semiquantitative classification (SQC), which has been suggested to be more sensitive than the ISKDC classification in predicting outcomes, and/or the MEST-C score (14, 15).

Table 1 | The EULAR/PRINTO/PRES criteria for the diagnosis of IgA vasculitis (13).

Criterion	Glossary
Purpura (mandatory criterion)	Purpura (commonly palpable and in crops) or petechiae, with lower limb predominance, * not related to thrombocytopaenia
1. Abdominal pain	Diffuse abdominal colicky pain with acute onset assessed by history and physical examination. May include intussusception and gastrointestinal bleeding
2. Histopathology	Typically, leucocytoclastic vasculitis with predominant IgA deposit or proliferative glomerulonephritis with predominant IgA deposit
3. Arthritis or arthralgias	Arthritis of acute onset defined as joint swelling or joint pain with limitation on motion Arthralgia of acute onset defined as joint pain without joint swelling or limitation on motion
4. 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

*For purpura with atypical distribution, a demonstration of an IgA deposit in a biopsy is required.

Table 2 | The International Study of Kidney Disease in Children (ISKDC) classification of renal biopsy.

ISKDC Grade	Description
Grade I	Minimal changes
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

1.1.6 Outcomes

Most children have a disease course that is self-limiting with symptoms resolving in the first month. The outcome at 2 years is excellent with 94% of children achieving full, spontaneous recovery (16). In around 25% of patients, recurrence of symptoms occurs and it has been suggested that patients >8 years, and those with nephritis, are more likely to have recurrent episodes (9). This most commonly occurs in the first six months of disease and patients and their families should be counselled to expect this. However, some children may experience either short- or long-term complications.

1.1.6.1 Short-term complications

Acute complications are mostly related to the gastrointestinal system and come in the form of abdominal pain (57%), intussusception (1.3-13.6%) or GI bleeding (1%) (9, 17).

1.1.6.1 Long-term complications

Renal disease makes up the majority of long-term complications (**Figure 2**). Around 40-50% of children will experience IgAV-N with 1-2% developing chronic kidney disease stage 5 (CKD 5) requiring renal replacement therapy (RRT) (18).

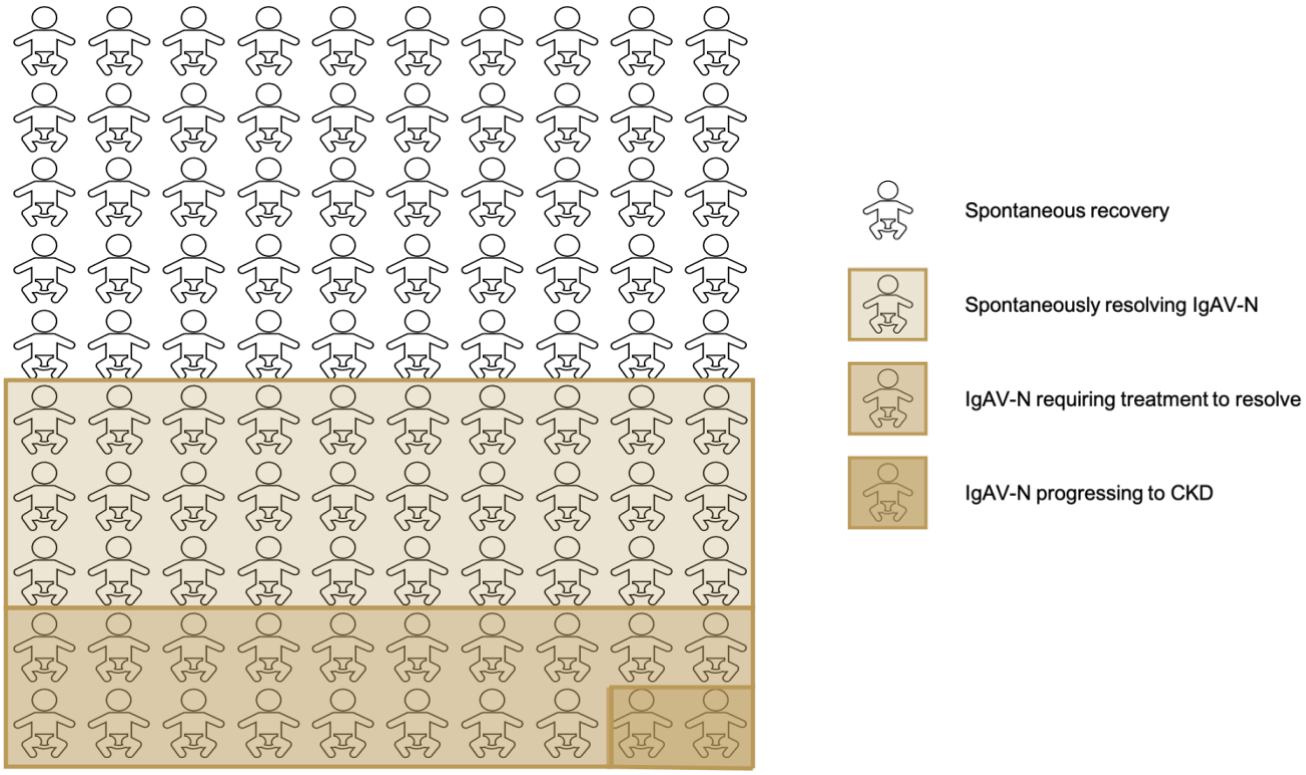
1.1.7 Management

There is a striking lack of evidence and no standardised treatment algorithms in IgAV. The European initiative, SHARE, aimed to enhance care for children with rheumatological conditions (12). In 2019, nineteen recommendations for the treatment of IgAV were made and categorised into three themes: analgesia, use of corticosteroids, and IgAV-N.

Management is supportive in the large majority of patients, with paracetamol and ibuprofen frequently prescribed to manage abdominal and joint pain/swelling. NSAIDs however, are contraindicated in patients with evidence of significant IgAV-N. Whilst treatment of the rash is usually unnecessary, in patients with severe, unremitting cutaneous manifestations, smaller studies have suggested the benefit of using oral prednisolone (19-21). Similarly, in patients with arthralgia/arthritis which doesn't respond to pain relief, corticosteroids may have a role (22).

Abdominal pain is usually short-lived and often doesn't require intervention other than adequate analgesia. However, severe abdominal pain, GI bleeding and intussusception all require further management, either medical in the form of oral or intravenous (IV) corticosteroids or surgical intervention (23).

Figure 2 | The predicted outcomes of children with IgAV. Of 100 children with IgAV, 50 would fully and spontaneously recover; 30 would develop renal involvement which would spontaneously resolve; 18 would have renal involvement needing treatment; and 2 would develop renal failure.

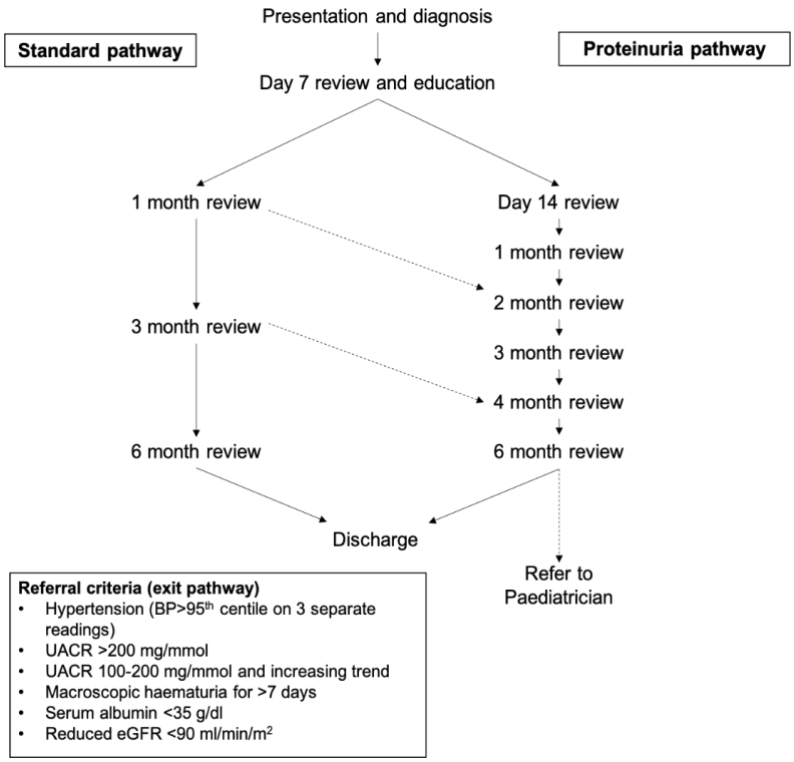


Management of IgAV-N is currently based on recommendations due to the lack of evidence: the Kidney Disease Improving Global Outcomes (KDIGO) glomerulonephritis guidelines and the SHARE initiative have made some proposals (12, 24). The KDIGO guidelines suggest the use of angiotensin-converting-enzyme inhibitors (ACE-i) or angiotensin receptor blockers (ARBs) in IgAV-N with oral corticosteroids as a second-line option (24). Importantly, repeated evidence has suggested that early intervention with corticosteroids should not be used to prevent the development of IgAV-N and this is echoed in the KDIGO guidelines (24-26). The SHARE initiative suggests that patients with IgAV-N should be categorised into mild, moderate, or severe nephritis (12). These categories are based on proteinuria, eGFR and biopsy findings, which then dictate treatment choices. Oral prednisolone should be used first-line for mild IgAV-N and commonly azathioprine, mycophenolate, cyclophosphamide, or pulsed methylprednisolone are used as a second-line therapy or as an adjunct in more severe IgAV-N. Treatment decisions are often made based on the opinion and experience of the managing paediatric nephrologist (12).

1.1.8 Follow-up

Although there are no international guidelines for the follow-up of IgAV patients, the literature suggests that all patients with a new diagnosis should have at least a 6-month period of screening for nephritis (1). The Alder Hey Henoch Schonlein Purpura nurse led Pathway was developed and published in 2012 and is used by many centres nationally and internationally as a framework for renal monitoring (**Figure 3**) (1). This pathway consists of serial blood pressure (BP) measurements and urine dipsticks. If patients have no signs of renal involvement at presentation, they can follow the "standard pathway" and can be discharged after checks at 1, 3 and 6 months without any positive findings. If any abnormalities are highlighted at review or patients have abnormalities at presentation, patients start on the proteinuria pathway, which measures BP and urine at day 14 and subsequently at 1, 2, 3, 4 and 6 months.

Figure 3 | A proposed example of renal monitoring for newly diagnosed IgAV (1).



1.2 Methods of measuring disease activity

Disease activity generally refers to aspects of a patient's disease that may be reversible. This can be distinguished from disease severity which assesses the extent of damage. A disease activity measure aims to quantify, particularly in rheumatology, the inflammatory process of the disease. There are several measures of disease activity that are often used in rheumatological conditions and may include traditional biochemical measurements, the quantification of inflammatory tissue, and the biopsychosocial consequences of the inflammatory tissue. Often, a combination of these are used to create tools to help objectively quantify, monitor and predict disease activity.

1.2.1 Qualitative methods

1.2.1.1 Rating scales

Physician-, parent- or patient-reported outcome measures can be used in different contexts. An example of a physician reported measure is a physician visual analogue scale (physician global assessment score) which provides a quick picture of disease activity in a patient and can be used to aid the validation scoring tools. They often come in the form of a 1-10 scale, with 1 being the least severe and 10 being the most severe disease. Because they are subjective and require expert clinical opinion, there is some question as to whether they are truly a gold standard measure when validating a scoring tool. Where one clinician may rate a person as 10/10 if they infrequently see patients with severe disease, another may only rate them a 7 or 8/10 if they commonly see severe complications.

1.2.2 Quantitative methods

1.2.2.1 Traditional markers

In rheumatology, the most commonly assessed markers of inflammation measured are C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR). They are both acute phase reactants with a high sensitivity and low specificity. In IgAV, neither CRP nor ESR have been found to be associated with gastrointestinal, renal, or joint involvement (27, 28). Autoantibodies are of huge importance in rheumatic diseases and when compared to traditional biochemical markers, they have much higher specificity and sensitivity (29). Some smaller studies found no association with IgA antineutrophil cytoplasmic antibody (ANCA) and IgA rheumatoid factor (RF) and IgAV (30, 31). No other markers have been found to be significant for IgAV.

1.2.2.3 Novel biomarkers

A biomarker is any outside, objective measure of a normal or pathogenic biological process which is accurate and reproducible (32). Over the last 20 years the use of reliable biomarkers has become established in the diagnosis and development of many diseases, ranging simply from blood pressure as a predictor of cardiovascular health to the use of serum cardiac enzymes such as troponins as an indicator for myocardial infarction. Biomarkers have many purposes, they can aid in early diagnosis,

be used as a surrogate endpoint for disease, allow personalised disease activity monitoring, and give clues to biological pathways. In clinical practice blood pressure, serum creatinine, proteinuria, haematuria, urinary albumin, and urine output have all been used as surrogate markers of renal injury however these are non-specific to IgAV nephritis and lack potential to improve the disease outcomes. In more recent literature sensitive markers of renal tubular injury have been indicated in IgAV-N such as N-acetyl-β-glucosaminidase (NAG), neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule-1 (KIM-1) and liver-fatty acid binding protein (L-FABP) as well as being indicated in other acute and chronic renal diseases. Other biomarkers such as monocyte chemoattractant protein-1 (MCP-1) and macrophage migration inhibitory factor (MIF) have been suggested in renal immune responses and inflammation, giving their potential use in conditions such as IgAV-N and lupus nephritis (33). For IgAV-N, we are lacking a reliable and reproducible surrogate marker with high sensitivity and specificity to accurately diagnose and predict the outcome of those patients with significant nephritis (34, 35). In the paediatric population, there is also an emphasis on discovering non-invasive biomarkers with urine being the most obvious biological substance.

1.2.3 Scoring tools

Scoring tools that encompass clinical, histological, and biochemical data are widely used in medicine and especially in rheumatology. Examples of these include the Disease Activity Score in 28 joints (DAS28) for rheumatoid arthritis (36), the Psoriatic Arthritis Response Criteria (PsARC) (37) and the Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI-2K) (38). To date, there have been no scoring tools developed specifically for IgAV.

1.2.3.1 The Paediatric Vasculitis Activity Score (PVAS)

The PVAS was developed and validated in 2012 as a modified version of the Birmingham Vasculitis Activity Score (BVAS), a scoring tool first validated in 1994 which is now used in clinical studies of adult vasculitis patients (39). The most updated version (BVASv.3) contains 56 features of active vasculitides which was reduced from 66 features in v.2 (40, 41). In this BVAS validation study, the tool was updated, and 20 basic level case reports were assessed by a group of 19 international experts; 40 advanced level cases were further assessed by 14 of these raters. 99 patients were also assessed by two raters on the same day to assess for inter-rater reliability which produced a high reproducibility (0.96, 95% CI 0.93-0.97). Scores from the latest version (v.3) were positively correlated with clinician treatment decision (0.66, 95% CI 0.59-0.72) and a strong correlation was found with the physician global assessment score in 307 patients with active vasculitis (r=0.91, 95% CI 0.89 to 0.93).

Dolezalova et al. in 2012 adapted 22 of the items included in the BVAS and 8 new items were added to create the PVAS (**Appendix 2**) (40). The revisions mainly included redefining clinical criteria to match

parameters suitable for paediatric patients, such as the definitions for weight loss, blood pressure, and eGFR. New additions to the tool included some cutaneous and cardiovascular manifestations, and bowel ischaemia. The now validated PVAS score includes 64 manifestations of various active vasculitides, each allocated to one of nine organ-based systems. Each item may be scored as new/worse or persistent. New/worse is defined as a manifestation that has developed or worsened in the past 4 weeks. Persistent is defined as any item present for longer than 4 weeks but less than 3 months. The new/worse scale is scored out of 63 and the persistent scale has a maximum score of 33 (40).

The PVAS validation study involved the prospective assessment of 63 patients with polyarteritis nodosa (28.6%), granulomatosis with polyangiitis (20.6%), Behçet's disease (17.5%), Takayasu arteritis (9.5%), cutaneous leukocytoclastic vasculitis (9.5%), unclassified systemic vasculitis (4.8%) and other vasculitides (9.5%) which included one patient with chronic relapsing-remitting IgAV. Face validity and content validity were assessed by a group of eleven paediatric rheumatologists with expertise in vasculitis. Inter-rater reliability was assessed by the scoring of 55 children by two independent assessors, with overall score agreement in 44/55 (82%) of patients. To evaluate convergent validity, a physician global assessment (PGA) was found to be strongly positively correlated with the PVAS scores (r=0.87, 95% CI 0.79 to 0.92, p<0.01). ESR and CRP were also compared to the PVAS scores to further assess convergent validity in 46 (r=0.37, 95% CI 0.09 to 0.6, p=0.01) and 48 (r=0.21, 95% CI −0.08 to 0.46, p=0.16) patients respectively. The PVAS has since been considered the gold-standard tool for measuring disease activity in children with vasculitis.

1.3 Aims

The overall aim of this thesis is to evaluate methods of measuring disease activity in IgAV using urine biomarkers and a disease-specific activity scoring tool.

2. A systematic review of urine biomarkers for children with IgA vasculitis nephritis

2.1 Introduction

All patients with IgAV should have a period of follow-up to screen for IgAV nephritis that currently consists of 6 months of periodic urinalysis to evaluate for haematuria or proteinuria and blood pressure monitoring, as surrogate markers of kidney injury (42). Earlier detection and management of kidney inflammation is believed to be the key to reducing the incidence of irreversible kidney damage in IgAV-N; a disease which currently contributes to 1-2% of all chronic kidney disease (CKD) (18). The gold standard practice for identifying nephritis is through histological analysis and therefore a kidney biopsy is conducted in those with signs of significant kidney inflammation on screening. However, the kidney biopsy is invasive and it may already reveal irreversible histological changes (43).

2.1.1 Aim

The aim of this chapter was to perform a comprehensive systematic literature review to identify promising traditional and novel urine biomarkers in children with IgAV.

2.2 Methodology

2.2.1 Study population

The inclusion criteria were paediatric participants (<18 years) of any sex and ethnicity, with a diagnosis of IgAV-N. A diagnosis of IgAV-N included any of the following: abnormal urinalysis; haematuria and/or a high urinary protein concentration within 6 months of the onset of rash; and/or a reduced estimated glomerular filtration rate (eGFR) in participants who had met the clinical diagnosis of IgAV (13). The exclusion criteria were studies that involved adult participants (>18 years) or participants who had other forms of nephritis or vasculitis (**Table *3***).

2.2.2 Intervention

The intervention of interest was biomarker assay evaluation in a urine sample.

2.2.3 Comparator

The study aimed to compare children with IgAV-N compared to children with IgAV and no nephritis (IgAV-noN) and/or healthy paediatric controls.

2.2.4 Outcome

There were two key outcomes of interest, the identification of traditional or novel biomarkers that are able to determine (i) the presence of nephritis as defined by each individual study and/or (ii) the severity defined in terms of the International Study of Kidney Disease in Children (ISKDC) classification histological grade or extent of proteinuria (43).

Table 3 | A summary of the inclusion/exclusion criteria in the form of a PICOS table.

	Include	Exclude
Patient population	Children (under 18) including neonates with a diagnosis of IgAV nephritis	Adults
Intervention	Urine sampling and biomarker assay	Other markers of nephritis including urinalysis and renal biopsy; skin biopsy; serum sampling
Comparator	Children without a diagnosis of IgAV, children with a diagnosis of IgAV *without* nephritis	Children with other forms of nephritis or vasculitis
Outcomes	Presence of urinary biomarkers, correlation of biomarkers with severity or duration of nephritis	Presence of serum biomarkers; markers present in the skin or kidney
Study design	Meta-analyses, RCTs, cohort studies, case-control studies, cross sectional studies, case series (N>5)	Systematic reviews, animal studies, case studies, any other secondary data
Overall decision	Include	Exclude

2.2.5 Study design

2.2.5.1 Data extraction

Using predefined methodology, this systematic review evaluated the current available literature. Four online databases, PubMed, Web of Science, Medline, and Scopus were used with the following terms which were created from five key concepts (**Table *4***): (((((((((neonat*) OR (adolescen*)) OR (infan*)) OR (child*)) OR (pediatric*)) OR (paediatric*)) AND ((((((immunoglobulin A vasculitis) OR (IgA Vasculitis)) OR (IgAV)) OR (Henoch Sch*nlein purpura)) OR (Henoch-Sch*nlein purpura)) OR (HSP))) AND (((((((nephritis) OR (renal injur*)) OR (kidney injur*)) OR (renal damage*)) OR (kidney damage)) OR (ckd)) OR (chronic kidney disease))) AND (urin*)) AND (biomarker*). The studies included were meta-analyses, randomised control trials (RCTs), cohort studies, case-control studies, cross-sectional studies and case series (n>5) that were all accessible in full text through the University of Liverpool, with at least an English abstract. Secondary data and animal studies were excluded, as well as papers with an original publication date before October 2000, allowing for a 20-year inclusion period. The reference lists of relevant literature were hand-searched to identify any additional eligible studies.

2.2.5.2 Data collection

From each included study, information was extracted on author, year of publication, study design, study population, definition of nephritis, type of sampling and laboratory technique, biomarkers assessed, and key findings. The relevant data was collected on a predesigned proforma by the primary author (CW). Where full English transcripts were unavailable, data was extracted from the English abstract.

Table 4 | The key concepts used to create the search terms.

Concept 1	Concept 2	Concept 3	Concept 4	Concept 5
P*ediatric* Child* Infan* Adolescen* Neonat*	Immunoglobulin A vasculitis IgA vasculitis IgAV Henoch-Sch*nlein Purpura HSP	Nephritis Renal injur* Kidney injur* Renal damage Kidney damage Chronic kidney disease CKD	Urin*	Biomarker*

2.2.5.3 Quality appraisal and statistical analysis

The "Appraisal tool for Cross-Sectional Studies" (AXIS) tool was used, which comprised of 20 questions to appraise and compare the quality of the literature (**Appendix *3***) (44). Novel biomarkers identified in more than one paper will be discussed in more detail. Those that have only been reported once will be to be summarised in a data table (**Appendix *4***). The results will be described in terms of traditional or novel biomarkers. A traditional biomarker is defined as any biological marker that is available in a routine clinical laboratory. A novel biomarker is one that is not routinely available in a clinical laboratory and deemed experimental (34). Where available, descriptive statistics will be presented as percentage male and a median age will be calculated using the available age data. Laboratory data will be presented as either a mean with standard deviation or as a median with range depending on the original publication. Area under the curve (AUC) will be presented to represent the strength of the biomarker and described as a value from 0-1.0 with a 95% confidence interval. In terms of biomarker strength, an AUC of ≤0.5 suggests no discrimination, 0.5-0.7 is unacceptable, 0.7-0.8 is considered acceptable, 0.8-0.9 is considered excellent, and ≥0.9 is considered outstanding (45). P-values <0.05 and a confidence interval which does not overlap 0 will be considered significant. As it was expected that the studies revealed would be heterogeneous, a meta-analysis was not conducted.

2.2.5.4 Ethical approval

Ethical approval was not necessary for the performance of this review, as per the National Health Service Research Authority, as it involved secondary review of existing literature.

2.3 Results

2.3.1 Data extraction

The search took place in September 2020 and yielded 121 papers. A total of 65 duplicates were removed leaving 56 titles eligible for abstract screening. Of these, 26 papers were eligible for full text review. After full text review, 11 were included in the systematic review. A second, independent reviewer (AT) repeated the search, at a time point 1 month later, to identify papers and determine whether the studies met the inclusion criteria; 128 papers were retrieved and after deduplication, two additional papers were identified that met the inclusion criteria, producing a total of 13 papers (**Figure 4**). No further eligible papers were discovered in searching the reference lists.

2.3.2 Participants

A total cohort of 2,446 children were included in this systematic review from 13 studies. The median age of the entire cohort was 7.9 years and 51% were male. Data on sex was not available in one study (46). Median or mean age was not available in two papers (46, 47) and age ranges could not be calculated due to the heterogeneity of the papers in presenting demographic data.

The participants comprised of 1,236 children with IgAV-N (48% male, median age 8.0 years), 449 children with IgAV-noN (52% male, median age 7.0 years), and 761 healthy paediatric controls (52% male, median age 7.9 years) (**Table *5***). The publication dates spanned from 2011-2020 (48-51) and included both longitudinal (48, 50, 52-55) and cross-sectional studies (46, 47, 49, 51, 56-58). The majority of the papers were published from China (46, 49-53, 55, 57-59), and three studies were from Poland (48), France (56) and Mexico (47).

2.3.3 Quality appraisal

The quality appraisal produced a good median AXIS score of 16/20 (range 14-17) (**Appendix *5***). One study was excluded from the quality assessment as it was not available in full text in English and there was insufficient detail in the abstract (50). Those studies with lower AXIS scores were mostly due to small sample size, single site recruitment, and no mention of study limitations.

2.3.4 Identified biomarkers

A total of 23 urine biomarkers were discovered that had been reported to be associated with IgAV-N; 20 were novel and 3 considered traditional biomarkers (). Increased urinary protein concentration was the only traditional urine biomarker identified and had been measured using 24-hour urinary protein (24h-UPRO) values, urinary protein:creatinine ratio (U-PCR) and urinary albumin concentration (Malb). There were 5 novel urine biomarkers that had been reported more than once and thus described in more detail, these were: beta-2 microglobulin (β2-MG), kidney injury molecule-1 (KIM-1), monocyte chemoattractant protein-1 (MCP-1), N-acetyl-β-glucosaminidase (NAG) and urinary angiotensinogen (UAGT) (**Appendix *6***).

Figure 4 | The search and screen process. The systematic literature search was performed on 4 databases and returned 121 papers. 56 papers were identified after deduplication. After screening by initial and a second independent person, a total of 13 studies were included in the systematic review.

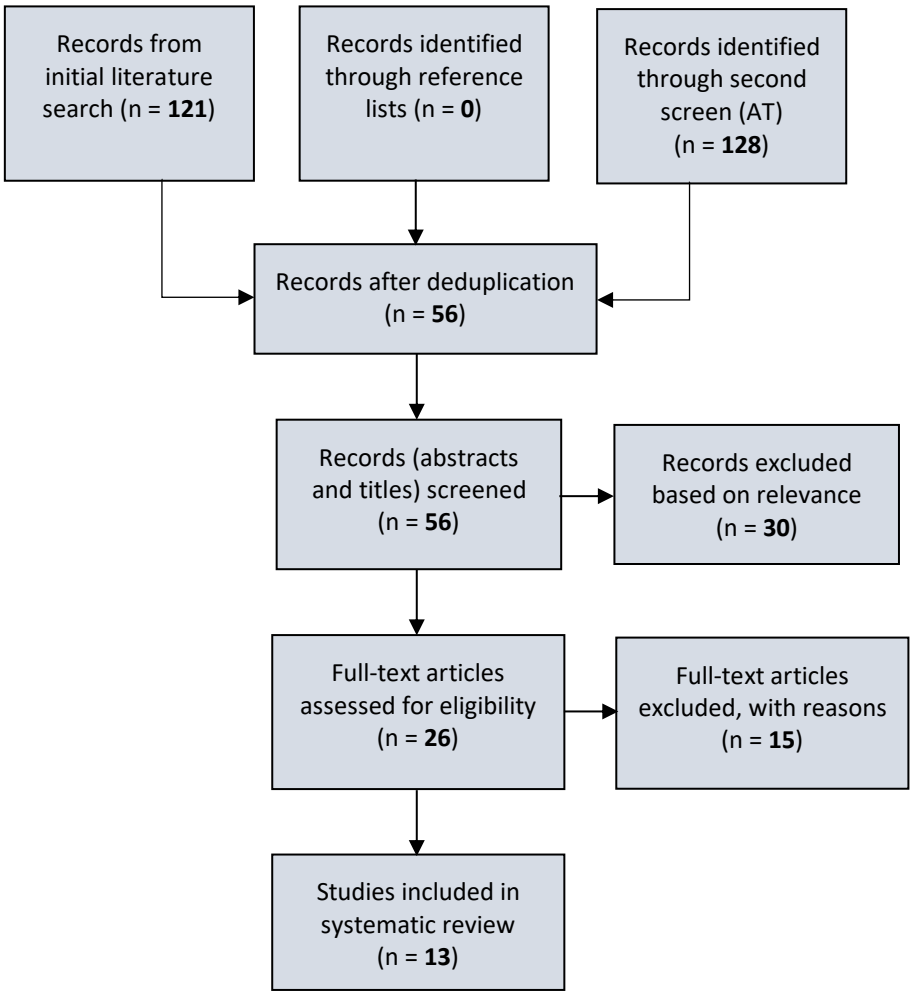


Table 5 | The characteristics of the cohorts identified in the systematic review.

Parameters	IgAV-N group (n = 1236)	IgAV-noN group (n = 449)	Control group (n = 761)
Male, number (%)	588 (48)	232 (52)	395 (52)
Age, median	8.0	7.0	7.9

2.3.5 Traditional biomarkers

2.3.5.1 Urinary protein concentration

(i) Presence of nephritis: As expected, the 24h-UPRO was significantly increased in children with biopsy proven IgAV-N (n=694) compared to healthy controls (n=400, p<0.01). In a second paper, the urine Malb concentration was significantly increased in the IgAV-N group (n=37, 108.00 (56.10-1800.00) mg/L) compared to both healthy controls (n=37, 8.30 (6.05-11.00) mg/L, *p*<0.05) and the IgAV-noN cohorts (n=34, 10.75 (6.65-16.78) mg/L, p<0.05). The control group was not significantly different to the IgAV-noN patients (*p*>0.05) (60).

(ii) Severity of nephritis: Importantly, differences could be seen within the IgAV-N cohort when comparing histological grades I and IIa versus IIb, IIIa and IIIb (all p<0.01). The AUC value was 0.77 for 24h-UPRO as a biomarker in distinguishing histology grades IIb, IIIa and IIIb. UPCR was also evaluated when assessing the severity of nephritis producing an AUC value of 0.73 (57). Malb positively correlated with the grading of IgAV-N (n=45, *p*<0.05) producing averages of 101.70±61.30, 367.8±157.01 and 654.9±275.1 mg/L for grades I, II and II respectively, with excellent AUC values for histological comparison (grade I vs II AUC 0.95, 95% CI 0.87-1.00; grade II vs III AUC 0.81, 95% CI 0.66-0.95; grade I vs III AUC 0.98, 95% CI 0.94-1.00) (46).

2.3.6 Novel biomarkers

2.3.6.1 Urinary beta 2-microglobulin (β2-MG)

(i) Presence of nephritis: One paper found that urine β2-MG was significantly increased in IgAV-N patients (n=37, 0.37 (0.18-1.02) mg/L) compared to both healthy controls (n=37, 0.11 (0.07-0.14) mg/L) and IgAV-noN (n=34, 0.14 (0.10-0.19) mg/L, all *p*<0.05) (60). Qin et al. reported statistically significantly increased urinary concentration of β2-MG in children with IgAV-N (n=66, 348.31±88.23 mg/L) compared to children with IgAV-noN (n=68, 92.76±36.49 mg/L, *p*<0.05) and both cohorts had urine concentrations much greater than the paper above (61).

(ii) Severity of nephritis: Another paper (IgAV-N, n=45) compared urinary β2-MG with the histological grades, grouped according to the ISKDC classification (43). They found that urinary β2-MG was statistically significantly increased in all groups (*p*<0.05) with no statistical difference between the histological classifications (46). Zhang et al. explored urinary β2-MG in predicting irreversible kidney damage (defined as histological changes according to the ISKDC criteria) and reported a suboptimal AUC at 0.49 (95% CI = 0.35-0.63, *p*=0.89) (55).

2.3.6.2 Urinary kidney injury molecule-1 (KIM-1)

(i) Presence of nephritis: This was reported as a potential biomarker in two studies. Dyga et al. found that KIM-1 was statistically significantly increased acutely in all IgAV patients (n=29, 30.5 (28.8-36.6) pg/mL) when compared to the controls (n=34, 15.1 (11.9-17.3) pg/mL, p<0.005) but there was no significant difference between IgAV-noN (n=18, 30.4 (28.8-33.7) pg/mL) and IgAV-N (n=11, 30.5 (26.7-37.1) pg/mL). Urinary KIM-1 concentrations decreased over time in both IgAV-N and IgAV-noN (48). Zhang et al. found the contrary with mean urinary KIM-1 concentrations significantly increased in IgAV-N (n=32, 2489.72±1098.30 pg/mL) compared to IgAV-noN (n=27, 1142.15±336.42 pg/mL, p<0.05) and healthy controls (n=16, 388.75±39.32, p<0.05). The AUC for KIM-1 in predicting nephritis was outstanding at 0.93 (95% CI = 0.88-0.99, p<0.05) (55).

(ii) Severity of nephritis: A positive correlation between urinary KIM-1 levels and histological grade or total urine protein was found in one paper studying 32 patients with IgAV-N, 27 with IgAV-noN, and 16 healthy controls (r = 0.671, p<0.01) (55). Another paper found no statistical difference in KIM-1's ability to distinguish disease severity (48).

2.3.6.3 Urinary monocyte chemoattractant protein-1 (MCP-1)

(i) Presence of nephritis: This was found to correlate with IgAV-N in two studies, reporting 447 children. Fuentes et al. reported a statistically significantly increased urinary MCP-1/Cr concentration in the IgAV-N cohort (n=57, 693 pg/mg) compared to IgAV-noN (n=27, 269 pg/mg) and healthy controls (n=25, 191 pg/mg, both p<0.01) (47). Wang et al. also found urinary MCP-1 to be significantly increased in IgAV-N (n=126, 311.82±151.72 pg/mL) compared to IgAV-noN (n=135, 73.09±27.48 pg/mL, p<0.01) and the healthy controls (n=84, 69.37±22.81 pg/mL, p<0.01). Urine MCP-1 concentrations increased in parallel with the degree of urinary protein concentration (62).

(ii) Severity of nephritis: One paper found the AUC for MCP-1 predicting nephritis was excellent (AUC 0.83 95% CI = 0.73-0.92, p<0.01) (47).

2.3.6.4 Urinary n-acetyl-beta-glucosaminidase (NAG)

(i) Presence of nephritis: Zhang et al. also found increased urinary NAG concentration in IgAV-N (n=32, 24.95±18.07 U/L) compared to IgAV-noN (n=27, 12.37±7.35 U/L, p<0.05). There was no difference between IgAV-noN (n=27) and healthy controls (n=16, 5.59±1.97 U/L, p>0.05). The AUC for urinary NAG in distinguishing patients with nephritis was excellent (AUC 0.8 95% CI 0.72-0.92, p<0.01) (55).

(ii) Severity of nephritis: An et al. evaluated urinary NAG in biopsy-proven IgAV-N (n=45). The concentrations correlated with increasing histological grade: 8.78±4.88 U/L in patients with

grade I IgAV-N, 23.01±13.31 U/L in grade II and 45.01±24.34 U/L in grade III. The differences were statistically significant (p<0.05). The AUC in predicting the histological grades were excellent for Grade I v II (AUC 0.84 95% CI 0.67-1.00); outstanding for grade I vs III (AUC 0.96 95% CI 0.89-1.00); acceptable grade II vs III (AUC 0.76 95% CI 0.59-0.93) (46).

2.3.6.5 Urinary angiotensinogen (UAGT)

(i) Presence of nephritis: Ma et al. compared IgAV-N (n=14), IgAV-noN (n=28) and healthy controls (n=23). UAGT/Cr was significantly increased in IgAV-N compared to IgAV-noN and healthy controls (p<0.05). This paper was unavailable in full text in English so limited data was extracted from the abstract only (50). Another paper by Mao et al. subdivided patients with IgAV-N and described an acute increase in UAGT in IgAV-N patients with a high urinary protein concentration (n=13, 32.02±3.95 μg/g) compared to both IgAV-noN (n=51; 17.26±2.6 μg/g) and IgAV-N with only haematuria (n=43, 19.70±2.21 μg/g, p<0.01) (53). This finding remained even during the convalescent phase where UAGT concentrations remained increased in the IgAV-N with a high urinary protein concentration compared to the IgAV-noN (25.31±4.11 μg/g vs 15.14±3.81 μg/g, p<0.01) and the IgAV-N with haematuria (25.31±4.11 μg/g vs 17.28±3.62 μg/g, p<0.01). The difference in concentration during the convalescent phase between the IgAV-noN and IgAV-N with haematuria was not significant (53).

(ii) Severity of nephritis: No studies assessed UAGT to determine the severity of nephritis.

2.3.7 Other biomarkers

2.3.7.1 Presence of nephritis

Neutrophil gelatinase-associated lipocalin (NGAL) and liver-fatty acid binding protein (L-FABP) were evaluated in one study. The concentration of urine NGAL was 61.1 (49.8-72.4) ng/mL in IgAV-N compared to 59.9 (38.9-73.9) ng/mL in IgAV-noN and 21.9 (19.9-27.7) ng/mL in the healthy controls. No significant difference was found to differentiate IgAV-N from IgAV-noN. However, levels were significantly higher in all IgAV patients when compared to the control patients (p<0.001). A similar pattern was seen with urinary L-FABP; concentration was lowest in the controls (4.5 (3.1-6.0) ng/mL) and was significantly lower than all IgAV children (p<0.001). However, no difference was found between the IgAV-N (11.6 (10.9-14.5) ng/mL) and the IgAV-noN patients (11.7 (10.5-14.0) ng/mL). Both NGAL and L-FABP were significantly lower at follow-up (p<0.001) but all IgAV patients still had elevated levels when compared to the healthy control children (48).

Integrin beta-1 (ITGB-1) and tenascin were found to be significantly lower in children with IgAV-N (n=30) compared to the healthy children (n=29, p<0.05) in one study. However, only tenascin was found to be significantly different in the IgAV-N and IgAV-noN groups (p=0.005). ITGB-1 was not

significantly lower in IgAV-N compared to IgAV-noN (p=0.508). No raw data for these biomarkers were provided, however the graph representing the results suggests tenascin is more significant than ITGB-1 (49).

Fibroblast specific protein-1 (FSP-1)M and thrombin were assessed as biomarkers for the detection of nephritis. FSP-1 was found to be significantly greater in IgAV-N when compared to both the IgAV-noN and healthy control groups (p<0.05). Urine thrombin was significantly raised in all IgAV patients compared to controls (p<0.05) but was unable to significantly differentiate the nephritis from those without (50).

Pillebout et al. measured urinary IgA/Cr and IgM/Cr and found it was significantly raised in children with IgAV-N (n=33, 1.4±0.3 and 0.2±0.2 respectively) compared to the IgAV-noN cohort (n=17, 0.1±0.0 and 0.0±0.0, p<0.0001) and healthy controls (n=21, 0.1±0.0 and 0.0±0.0, p<0.0001). IgG/Cr and the Igλ/IgK ratios were significantly higher in the IgAV-N group (4.9±1.2 and 1.4±0.4 respectively) when compared to the IgAV-noN cohort only (0.4±0.0 and 0.6±0.2, p <0.01). IL-6/Cr and IL-8/Cr were both increased in the urine in patients with IgAV-N (4.5±1.1 and 10.9±2.4 respectively) when compared to the IgAV-noN group (0.6±0.2 and 1.6±0.5, p <0.0001) and the healthy controls (0.0±0.1 and 2.0±0.6, p <0.01). IL-2/Cr was found to be significantly increased (0.8±0.1) only when compared to the IgAV-noN children (0.2±0.1, p<0.01) and not when compared to the controls (56).

A prospective longitudinal study measured the concentration of macrophage migration inhibitory factor (MIF) in the urine children with IgAV-N (n=35), IgAV-noN (n=41) and healthy controls (n=32). Urinary MIF was highest in those with IgAV-N (3.17±1.29 ng/mL) and significantly higher than IgAV-noN (1.02±0.58 ng/mL, p<0.05) and the controls (0.87±0.34 ng/mL, p<0.05). There was no statistically significant difference between IgAV-N and IgAV-noN (58).

Urine matrix metallopeptidase 9 (MMP-9) and tissue inhibitor of metalloproteinase 1 (TIMP-1) were measured in children with IgAV-N (n=66), IgAV-noN (n=68), and healthy controls (n=60). Urinary MMP-9 was significantly higher in IgAV-N (54.11±15.74 ng/mL) compared to both IgAV-noN (30.83±8.73 ng/mL, p<0.05) and the controls (23.60±4.59 ng/mL, p<0.01). Similar patterns were seen with urinary TIMP-1, with the IgAV-N cohort (155.02±48.09 ng/mL) showing significantly higher concentrations than IgAV-noN (121.38±28.28 ng/mL, p<0.05) and the healthy controls (108.28±18.85 ng/mL, p <0.01). Again, the ratio of MMP-9/TIMP-1 was significantly higher in the IgAV-N children (0.34±0.12 ng/mL) compared to the IgAV-noN cohort (0.25±0.09, *p<0.05)* and the healthy volunteers (0.22±0.08, p<0.01).

No significant differences in either of these biomarkers were seen between the IgAV-noN cohort and the controls (61).

2.3.7.2 Severity of nephritis

Urinary transferrin (Tfr) levels were significantly different between histological grades in a cohort of children with biopsy-proven IgAV-N (n=45), with the lowest concentration in grade I patients (7.92±6.55 mg/L), 42.64±31.63 mg/L in grade II patients and highest in grade III (78.21±43.73 mg/L, all $p<0.05$). For grade I vs II, AUC was 0.95 (95% CI = 0.87-1.00), 0.76 (95% CI = 0.59-0.93) for grade II vs III, and 0.99 (95% CI = 0.98-1.00) comparing grade I to III (46).

The IgAV-N (n=68) cohort in one study were also divided into three subgroups: mild, moderate and severe proteinuria (groups A, B and C respectively). Urinary MMP-9 was significantly greater in group C when compared to group A (97.60±29.10 vs 45.48±17.59 ng/mL, $p<0.001$) and group B (97.60±29.10 vs 57.98±11.64 ng/mL, $p<0.05$). A similar finding was revealed for MMP-9/TIMP-1, with group C showing significantly raised levels compared to group A (0.59±0.11 vs 0.30±0.07, $p<0.01$) and group B (0.59±0.11 vs 0.36±0.09, $p<0.05$). Urinary TIMP-1 concentration was not significantly different between these groups (61).

2.4 Discussion

This chapter aimed to identify potential urine biomarkers in predicting the presence and/or determining the severity children diagnosed with IgAV-N. Using a predetermined systematic evaluation, we have reported a cohort of 2,446 children, including 1,685 children with IgAV, from 13 papers. These data identified 4 promising novel biomarkers within the literature that may be significantly associated with IgAV nephritis: KIM-1, MCP-1, NAG and UAGT (47, 48, 50, 53, 55, 58). One biomarker, β2-MG, although frequently studied, did not perform well in the literature available (46, 55, 60). Additionally, we have reviewed the performance of the traditional urine biomarker, proteinuria, either reported as Malb, 24h-UPRO or U-PCR, and discovered a further 18 markers that were less frequently reported but may have potential future utility in this disease.

From our findings it can be concluded that the traditional biomarker of proteinuria performed best when evaluated using microalbuminuria with excellent AUC values (AUC 0.81-0.98) in determining the grade of histological inflammation in IgAV-N. Proteinuria measured in 24-hour values or as protein: creatinine ratios only produced acceptable AUC values (0.73-0.77).

In addition to identifying potential markers of disease presence and/or severity, understanding the mechanism of action of the novel biomarkers may reveal insight into the pathophysiology of IgAV-N. The most promising novel biomarkers will be discussed in more detail.

2.4.1 Urinary kidney injury molecule-1 (KIM-1)

KIM-1 is a type 1 transmembrane protein that is absent in the normal kidney and upregulated in tubular injury (63). KIM-1 is not expressed in other organs, so it is exceptionally specific to kidney injury (63). It is recognised as a biomarker in acute tubular necrosis and allograft nephropathy where it has been found to correlate with the degree of insult (64-66). It has not yet been reported in the histology for IgAV-N, but urinary KIM-1 has also been suggested to correlate with the degree of tubulo-interstitial injury in adults with IgA nephropathy, suggesting a role in IgA related renal disease (67, 68). This review included one paper, with a small sample size, that found no clear relationship between KIM-1 concentration and IgAV-N but it did demonstrate that it reduced over time suggesting some relationship with disease activity (48) and a larger study by Zhang et al. reported an outstanding AUC (0.93) for KIM-1 in its ability to identify IgAV-N (69). The potential association of increased KIM-1 in this disease suggests there may be a larger role for tubulo-interstitial inflammation than previously acknowledged (70).

2.4.2 Urinary monocyte chemoattractant protein-1 (MCP-1)

MCP-1 is an inflammatory chemokine that recruits monocytes and macrophages to sites of injury. It is mainly produced by these leukocytes, as well as endothelial, fibroblasts, smooth muscle, and astrocytic cells (71). MCP-1 was reported to be a potential diagnostic and predictive biomarker in two papers (47, 62). It was able to distinguish between IgAV-N and IgAV-noN and it was significantly associated with endocapillary histological changes. The AUC (0.83) for MCP-1/Cr was excellent. Urinary MCP-1 has previously been studied in adults with IgA nephropathy and in lupus nephritis, therefore it may have a key role in glomerular inflammation (72).

2.4.3 Urinary n-acetyl-beta-glucosaminidase (NAG)

The lysosomal enzyme NAG is found in many body tissues, but it is found in particularly high concentrations in the proximal renal tubular cells. NAG may be released into the urine via exocytosis or, more commonly during renal injury causing proximal tubule leakage (73). Urinary NAG has been described in patients with acute kidney injury and more recently in diabetic nephropathy, however there are few studies in IgA mediated renal diseases (74-76). Our review found urinary NAG as a predictive biomarker, able to accurately correlate with the degree of histopathology in IgAV-N (46) and detect the patients with IgAV-N from those without nephritis (69). The AUC (0.82) in detecting nephritis was excellent (77) and it again highlights the need to explore the importance of tubular

inflammation in IgAV. Tubular markers may be evident due to tubular damage leading to urinary release of these proteins as a downstream result of glomerular damage or from direct tubular involvement. The latter may be more likely as tubulointerstitial components have recently been added to proposed histological scoring classification systems for IgAV-N due to their better correlation with clinical outcomes, supporting the finding that the tubulointerstitial region is of importance in this disease (15).

2.4.4 Urinary angiotensinogen (UAGT)

Angiotensinogen (AGT) is the only known passive substrate of the renin-angiotensin-aldosterone system (RAS) and is primarily synthesised and secreted by the liver. Ordinarily, serum AGT is too large to pass through the glomerulus, however it is possible that in a defected glomerulus the protein could be filtered through and be present in the urine. Alternatively, it has been found that proximal tubule cells can produce AGT and secrete it directly into the lumen (78). Over-activation of the RAS is associated with inflammation and consequently there may be a local increase in the vasoconstrictor angiotensin II which is implicated in the pathological process. This requires up-regulation of AGT as a substrate by the proximal tubule cells, increasing AGT accumulation and hence urinary loss (53, 79). In IgAV, activation of the RAS is described and a deletion polymorphism of the angiotensin converting enzyme gene has been shown to predict proteinuria (80). During the acute phase, we found that UAGT was significantly increased in children with IgAV-N compared to those without nephritis (50). Overall, UAGT may be a promising biomarker and its presence may give insight into the important role of the RAS in this disease and support the treatment benefit of RAS inhibition.

2.4.5 Future use of urinary biomarkers for IgAV-N

Nephritis is the main long-term complication of IgAV and there is currently no way to predict and identify which children may get irreversible CKD. Improved markers of kidney inflammation could help to identify children who are at a high risk of disease progression and may provide insight into the inflammatory biology driving this disease allowing targeted treatment. Albuminuria performed well in the limited studies available for our evaluation and our review suggests that there is potential for novel biomarkers to act as adjuncts to current practice.

2.4.6 Limitations

Limitations of this study include some studies being small and the heterogeneous nature of the papers regarding descriptive statistics, definition of nephritis, and type of sampling, methodologies, outcomes, and data presentation made comparisons challenging. This review has identified the need for standardisation of biomarker evaluation in this disease to allow systematic comparison in the future. Some papers had missing data and one was only available in abstract form in English. The majority of these studies were cross sectional in design so future longitudinal studies are needed to

evaluate how the biomarkers change with the course of disease over time. Finally, most of the papers included in our review were from China and the relevance of ethnic variation of the expression of urinary biomarkers is currently unknown.

2.4.7 Core outcome measures

The Standardising Outcomes in Nephrology (SONG) Initiative has recognised the need for core outcome measures when undertaking renal research and this is a big advancement for nephrology as a speciality (81). This chapter suggests the need for definitions and a core outcome set for biomarker evaluation in renal diseases. Simple measures such as using medians for non-parametric data e.g., age, should be implemented to standardise reporting in trials. More specifically, one method of urine sampling e.g., first morning sample or 24-h collection should be suggested for use in all research, as well as a standardised method of biomarker assay which would likely be enzyme-linked immunosorbent assay (ELISA) due to its low cost and wide accessibility. Currently, a meta-analysis of published data was not possible with the heterogeneous reporting. A standard definition of nephritis is required, and this should be based on histological grading and/or clinical features. However, since the newer studies introducing alternative histological classifications such as the MEST-C and SQC, these may need evaluating and agreement on which is best to use (14, 15).

2.5 Conclusion

Overall, this chapter has suggested that there are promising urine biomarkers for IgAV-N and some of these originate from the tubulointerstitial region and may give clues into the pathophysiology of the disease, such as RAS activation. In order to assess their potential as adjuncts to clinical practice, more preclinical studies are needed, including longitudinal biomarker analysis with clinical correlation.

3. The development and preliminary validation of the IgA-VAS scoring tool

3.1 Introduction

Currently, the Paediatric Vasculitis Activity Score (PVAS) is used in clinical vasculitis trials to objectively monitor disease activity in children with a diagnosis of vasculitis. The PVAS includes 64 items grouped into 9 categories, some of which are irrelevant to the manifestations of IgAV, such as ear, nose, and throat (ENT), chest, and cardiovascular manifestations and therefore could lead to low overall scores and limited variability between patients making it difficult to accurately describe disease activity in IgAV. Considering the unchanging rates of IgAV-induced CKD in children over the last few decades, clinical trials which include objective descriptions of disease activity are urgently needed (82). A more specific tool would not only be more accurate in describing a patient's disease activity, but it would also allow better distinction between those who are the most acutely unwell and may require intervention. It would permit an objective measure for comparison in research studies, as well as better communication between health professionals.

3.1.1 Validity and reliability testing for the validation of a scoring tool

For a diagnostic test or tool to be accepted for general use, it needs to be both valid and reliable. Test validity is the extent to which a score or test result is accurate compared to the true value. Reliability is the extent to which a score or test result is consistent. This can be over time, across items and between researchers. Both validity and reliability have subtypes and for a tool to be considered 'validated for use', a range of validity and reliability should be undertaken. Test validity encompasses face validity, content validity, construct validity and criterion validity however it is also accepted to be one distinct unitary concept. Reliability generally comprises test-retest reliability, internal consistency and inter-rater reliability (83-86).

3.1.1.1 Face validity

Face validity is the extent to which a method measures what it intends to measure, in other words, at face value. Because it is subjective, it is a weak form of evaluating validity and is often used in combination with other measures.

3.1.1.2 Content validity

Content validity asks the question "does this test cover what it aims to cover?". For a test to produce valid results, it must cover all relevant aspects of the concept being measured.

3.1.1.3 Construct validity

Construct validity is "the experimental demonstration that a test is measuring the construct it claims to be measuring" (87). It usually involves an external measure of something that cannot otherwise be

quantified. To obtain good construct validity, the other indicator used to measure the construct need to be carefully researched and created based on currently accepted, relevant clinical knowledge.

3.1.1.4 Criterion validity

Criterion validity evaluates whether the test in question correlates with another criterion. This other criterion is often the current gold standard test or tool used in practice. This can take several different forms:

- *Concurrent validity* – When the criterion is tested at the same time as another criterion, this is referred to as concurrent validity.
- *Predictive validity* – This type of criterion validity is when the construct is measured once and then again in the future.
- *Convergent validity* – Convergent validity describes the degree to which two or more measures of construct correlate with each other. Theoretically these constructs should be related, and a high degree of convergent validity confirms this.

3.1.1.5 Test-retest reliability

Test-retest reliability is the degree to which a test or tool is consistent over time. An example of this is a measure of intelligence. A tool with a high test-retest reliability would produce the roughly same result for the same person, under the same conditions, week after week. This is typically measured using Pearson's coefficient.

3.1.1.6 Internal consistency

Another type of reliability is internal consistency which is measured when there are multiple items in a tool or test. It considers whether you would achieve the same result from the different parts of the test if they supposedly measure the same thing. For example, if in a survey a respondent ticked "agree" to the statement "I enjoy driving cars" and ticked "disagree" to "I dislike cars", there would be a high internal consistency. Internal consistency can be measured using Cronbach's alpha test.

3.1.1.7 Inter-rater reliability

The last type of reliability considers the agreement between two or more raters/observers when they perform the test or use the tool on the same individual. If both raters, ideally blinded to each other's results, produce the same score, a high degree of inter-rater reliability is achieved. The Cohen's kappa statistic is used to measure inter-rater agreement.

3.1.2 IgA-VAS

The IgA-VAS was created by compiling the possible features in the presentation, history, or examination of patients with IgA vasculitis and split into organ-system domains and its initial design was aimed to align with the PVAS (**Appendix *7***). The original design and a survey was distributed in January 2020 as part of a face, content and construct validity study performed by a previous student

(JM) to five research groups: The British Association for Paediatric Nephrology (BAPN); British Society of Paediatric Gastroenterology, Hepatology and Nutrition (BSPGHAN); Paediatric Emergency Research in the UK and Ireland (PERUKI); UK & Ireland Vasculitis Rare Disease Group (UKIVAS); and the British Association of General Paediatricians (BAGP). Data was collected on responder demographic, suitability and completeness of the tool, weightings for each component and any additional comments. A total of 33 people completed the survey. As part of face validity, the participants were asked "at first impression, does the IgA-VAS tool appear suitable to assess disease activity?", to which 27 (82%) of responders answered "yes". A total of 16 (50%) respondents answered "no" when asked whether they thought anything was missing from the tool and 11 (34%) respondents felt that something was missing. Many of these respondents left comments suggesting additions and improvements to the tool including adding testicular involvement, better definitions of pain management in terms of analgesia and histological findings. Participants were also asked to align each manifestation with a numerical weighting from 0-5. The findings were summarised by JM and the tool was subsequently updated as part of this thesis project.

3.1.3 Aims

The aim of this chapter is to perform further validation of a vasculitis activity scoring tool for IgAV (IgA-VAS) in terms of qualitative content validity, construct validity, criterion validity, and inter-rater reliability.

3.2 Methods

3.2.1 Patient cohort

To validate the IgA-VAS, disease activity was retrospectively assessed in a cohort of children presenting to a single centre: Alder Hey Children's Hospital, Liverpool UK. The inclusion criteria were: patients aged 0-18 years who attended Alder Hey Children's Hospital between 01 January 2015 to 31 December 2019 with a clinical and/or histological diagnosis of IgAV. Patients were identified by the information technology (IT) team using the International Classification of Diseases (ICD-10) coding system to compile a list of all the patients who had been diagnosed with IgAV in this period. A clinical diagnosis of IgAV was made by the receiving clinician at the time of attendance to hospital. Renal histology was graded according to the ISKDC criteria and any cutaneous histology would be described. Excluded patients were: >18 years of age at presentation, patients with no clinical diagnosis of IgAV, and patients with insufficient available data to score.

3.2.2 Data collection and definitions

Anonymised clinical data were recorded on a standardised data table which included patient demographics, manifestations of the disease, score from the visual analogue scale, treatment

decisions, whether a skin and/or renal biopsy was undertaken and patient outcome. Disease activity was scored using the IgA-VAS and the PVAS during the acute phase of the disease. Acute disease was defined as the presence of a feature or features that were new or worsened within the last four weeks. Therefore, data from 4 weeks prior to admission or presentation at Alder Hey was included, as well as the data from 4 weeks following the end of their episode of care. This included any data present over multiple admissions in this 8-week period. The highest values for systolic blood pressure, temperature, serum creatinine, urinary creatinine, and urinary albumin:creatinine ratio (ACR) were used in the data collection. Weight and height centiles were calculated using the World Health Organisation (WHO) Child Growth Standards growth charts. Hypertension was defined as a systolic blood pressure above the 95th centile for the child's age, sex and height (88). eGFR was calculated using the following calculation: $\frac{height\ (cm) \times 40}{acute\ creatinine\ (mg/mmol)}$ (89).

3.2.3 Handling missing data

Due to the retrospective nature of this study, it was likely there was missing data. Where weight or height data was not available, the value corresponding to the 50th centile was used as an imputation. Urine protein:creatinine ratio (PCR) was calculated from the ACR under the assumption that a U-PCR of 250mg/mmol = a U-ACR of 132.6mg/mmol (90). As 24h urinary protein excretion is not standard practice in paediatric clinical practice and is not performed routinely, it was presumed that an ACR>15.8mmg/mm Cr is equivalent to the 0.3g/day of proteinuria as a cut off value in the PVAS (91). Where a manifestation was not explicitly documented or an investigation was not performed, it was be presumed absent, and they scored 0.

3.2.4 Content validity

The IgA-VAS was amended based on the comments received from respondents of the previous survey. This included the addition of rarer manifestations of IgAV; more comprehensive definitions of analgesia used as a grading for abdominal pain; and better descriptions of renal involvement. As part of the content validity, participants were asked how they would weight each item on a scale from 0-5. Content validity was assessed qualitatively by the raters regarding suitability and ease of use when scoring patients.

3.2.5 Construct validity

A subgroup of 30 patients were selected using an online random number generator (92) to have additional scoring using a 1-10 physician visual analogue scale (**Figure 5**) by a Paediatric Speciality Grade 6 Trainee doctor (TD) who will be independent to the other raters. The physician visual analogue scale aimed to indicate overall disease activity and it was used to compare the total scores from both the IgA-VAS and the PVAS. Construct validity was measured by comparing both the PVAS and IgA-VAS with the physician visual analogue scale to assess for correlation.

3.2.6 Criterion validity

To assess for criterion validity, concurrent validity was evaluated. The PVAS was scored by each independent rater on the same day as the IgA-VAS at the same time point for the patient. For the purpose of this study, the participants included were only scored at the time of presentation and therefore the "persistent disease" weightings in the PVAS were not used.

3.2.7 Inter-rater reliability

Disease activity was scored by two independent raters blinded to each other's results. Raters scored the patients using both the IgA-VAS and the PVAS to assess inter-rater reliability. Rater 1 and rater 2 were an intercalating student doctor (CW) and Consultant Paediatric Nephrologist (LO) respectively. Both raters read the supporting instructions and were briefed on how to use each tool.

3.2.8 Statistical analysis

All statistical analyses were conducted using IBM Statistical Product and Service Solutions (SPSS) Statistics v27.0 and GraphPad Software Inc. Non-parametric, descriptive data was presented as a median with range and sex as percentage male. To describe organ involvement, values from rater 1 were used. The Cohen's kappa method was used to assess inter-rater agreement and the two-tailed Pearson's correlation was used to assess correlation between the IgA-VAS and PVAS and the visual analogue scale. Inter-rater reliability coefficient was interpreted as: <0.20=unacceptable, 0.20–0.39=poor, 0.40–0.59=good, 0.60–0.79=very good, and 0.80–1=excellent (93). A two-tailed Pearson's correlation coefficient which lies between ±0.5-±1.0 was indicative of a strong correlation, with values between ±0.3-±0.49 suggesting a moderate correlation and a value of <±0.3 implies a weak correlation. Any imputations in the data were included in the analysis. A p-value <0.05 was considered significant, as well as a 95% confidence interval that does not cross 0.

3.2.9 Ethical approval

According to the NHS Health Research Authority, this study was not considered research as it involved anonymous retrospective data collection for clinical purposes and therefore did not require ethical approval (see certificate, **Appendix 8**).

3.3 Results

3.3.1 Patient cohort

A total of 196 children were electronically coded as having IgAV between 01 January 2015 and 31 December 2019. Of these, 29 were incorrectly coded and a further 14 had insufficient electronic data,

leaving 153 eligible and included for retrospective scoring (**Figure 6**). From this cohort, 54% were male with a median age of 5.7 years (range 0.6-16.7, **Table *6***).

Figure 5 | The visual analogue scale used to score a subgroup of patients to assess for construct validity.

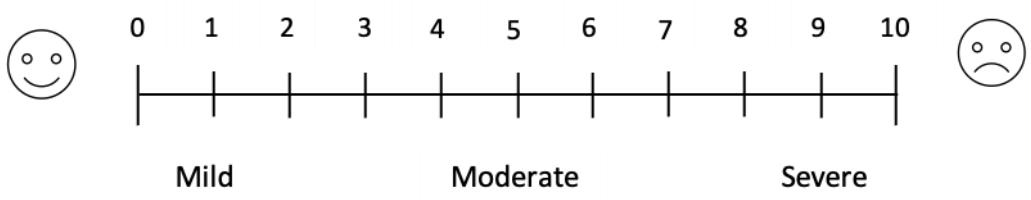


Figure 6 | The flow of identifying eligible patients who were retrospectively scored using the IgA-VAS and PVAS tools.

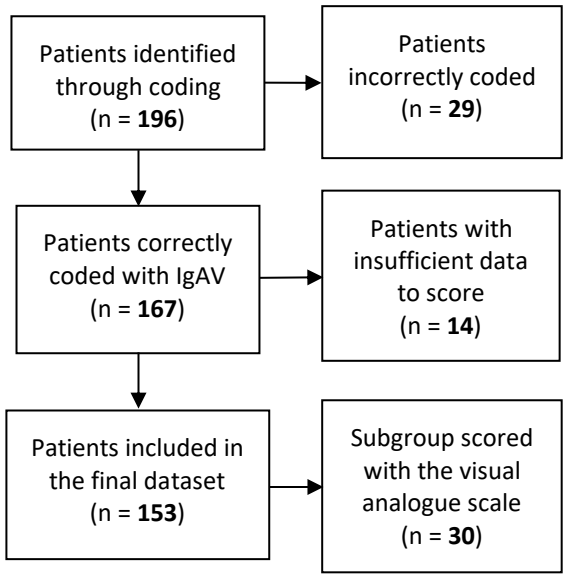


Table 6 | Cohort characteristics of the patients retrospectively scored with the IgA-VAS and the PVAS.

Parameter	Children with IgAV (n = 153)
Male, number (%)	83 (54)
Age, years, median (range)	5.7 (0.6-16.7)
Weight, kg, median (range)	20.7 (8.1-71.4)
Height, cm, median (range)	113.8 (65.0-181.1)
Systolic blood pressure, mmHg, median (range)	117 (84-191)
Temperature, °C, median (range)	37.5 (35.3-39.9)
Serum creatinine, µmol/L, median (range)	40.0 (20.0-116.0)
Urine creatinine, mmol/L, median (range)	8.6 (0.9-30.9)
U-ACR, mg/mm Cr, median (range)	5.5 (0.5-3642.0)
U-PCR, mg/mmol, median (range)	10.3 (0.9-6866.5)
eGFR, mL/min/1.73m^2, median (range)	117.4 (53.3-213.2)
Platelet count performed, number (%)	119 (78)
Renal biopsy, number (%)	9 (6)
Skin biopsy, number (%)	15 (10)
Cutaneous involvement, number (%)	148 (96.7)
Gastrointestinal involvement, number (%)	69 (45.1)
Musculoskeletal involvement, number (%)	95 (62.1)
Renal involvement, number (%)	89 (58.2)
Other involvement, number (%)	35 (22.9)

3.3.2 Missing data

18 (11.8%) children had no recorded weight and 88 (57.5%) had no recorded height. 12 (7.8%) had no temperature taken, 8 (5.2%) had no serum creatinine, 38 (24.8%) had no urine creatinine and 55 (35.9%) had no urine ACR.

3.3.3 Content validity

Following the 33 anonymised responses to the survey performed by the previous student, the IgA-VAS was updated (CW) to address these comments. With regards to proposed numerical weighting, some items had a clear clustering around one or two numbers whilst others were more evenly spread across different numbers. Where there was a clear majority, this weighting was used. In cases where the score was unclear, it was finalised by one of the raters (LO). The changes made to the IgA-VAS included adding items related to the distribution of cutaneous manifestations, defining the strength of analgesia needed to control pain, a wider range of items for describing renal involvement and a new domain involving rarer manifestations of IgAV (**Table *7***, **Appendix *9***). Following the scoring process, both raters compiled a list of the advantages, disadvantages, ease of use and suggested changes to assess the IgA-VAS and the PVAS (**Table *8***).

Table 7 | The additions and revisions made to the IgA-VAS following the content validity study.

Section	Modification	IgA-VAS item
Cutaneous	Additions	Distribution most common - legs, arms, buttocks
		Distribution common trunk, chest, feet
		Distribution uncommon palms
		Distribution rare face, head, neck
Gastrointestinal	Revisions	Ischaemic abdominal pain manageable with analgesia from step 1 of the WHO analgesic ladder (non-opioid analgesics and NSAIDs +/- adjuvants)
		Ischaemic abdominal pain requiring analgesia from step 2 of the WHO analgesic ladder (weak opioids +/- adjuvants)
		Ischaemic abdominal pain requiring analgesia from step 3 of the WHO analgesic ladder (strong opioids +/- adjuvants)
		Intermittent vomiting but tolerating oral diet
		Severe vomiting and not tolerating oral diet
		Melaena or gastrointestinal bleeding
Renal	Additions	Proteinuria with a urine PCR >250mg/mmol Cr (or equivalent)
		Persistent proteinuria (2+ or more) beyond 3 months
		Histological evidence of IgAV-nephritis
	Revisions	Estimated GFR 50-80 ml/min/1.73m^2
		Estimated GFR 15-49 ml/min/1.73m^2
		Estimated GFR <15 ml/min/1.73m^2
Other	Additions	Constitutional features (fever, weight loss, lymphadenopathy)
		Orchiditis (such as scrotal pain or swelling)
		Pulmonary haemorrhage
		Neurological involvement (headaches, encephalitis, or seizures)

Table 8 | A summary of the advantages and disadvantages of the IgA-VAS and the PVAS noted by the raters.

	Advantages	Disadvantages
IgA-VAS	Created specifically for children Captured abdominal and cutaneous involvement much more clearly than PVAS More specific to manifestations of IgAV	Difficult to know whether to score the worst sign/symptom or all Some individual criteria are signs, and some are symptoms Difficult to score fever in patients with intercurrent infection Abdominal domain missed off endoscopy findings e.g., intramural bleeding Renal domain felt similar to PVAS
PVAS	Already validated for use Clear definitions and instructions on how to use	Complex scoring process Doesn't distinguish arthritis from arthralgia No relevant cutaneous criteria other than purpura Domains 3, 4, 5, 6 and 9 were mostly irrelevant Abdominal domain only includes two relevant criteria: pain and bleeding

The retrospective scoring was done in February 2021 by two independent people. The median total scores for the IgA-VAS were 7/125 (range 2-31) and 5/125 (range 2-29) for rater 1 and rater 2 respectively. Median scores for the IgA-VAS subsystems for rater 1 and rater 2 respectively were 2/24 (range 0-12) and 2/24 (range 2-6) for cutaneous; 0/19 (range 0-14) and 0/19 (range 0-15) for gastrointestinal; 1/5 (range 0-4) and 1/5 (range 0-4) for musculoskeletal; 2/52 (range 0-24) and 0/52 (range 0-24) for renal; and 0/5 (range 0-5) and 0/5 (0-3) for other manifestations.

3.3.4 Construct validity

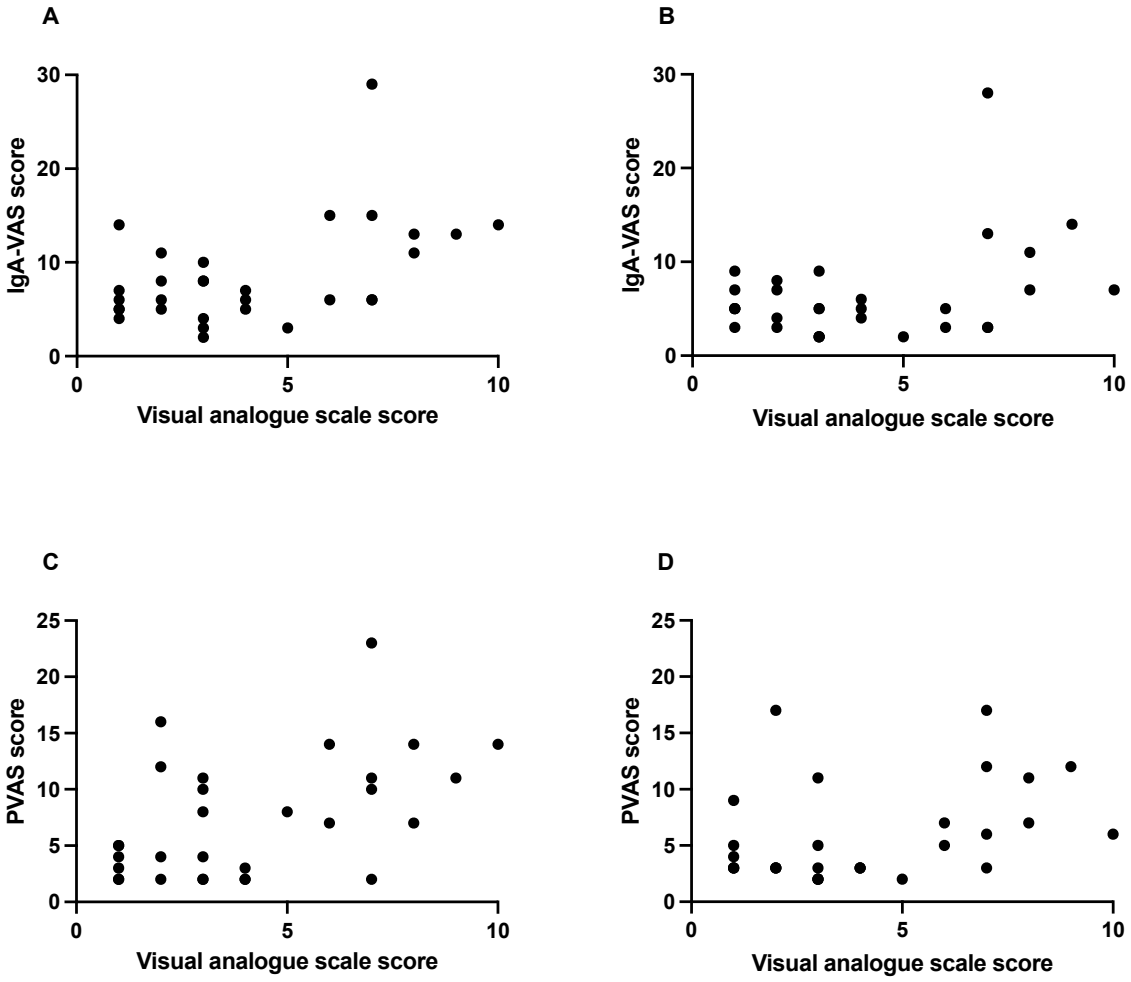
A subgroup of 30 patients were randomly selected to be scored with the physician visual analogue scale by an independent clinician (TD), of which 50% were male with a median age of 5.6 years (range 0.9-16.7 years). Other descriptive statistics can be found in **Table *9***.

Median physician visual analogue scale score for this subgroup was 3/10 (range 1-10/10). Scoring for the IgA-VAS by both raters was moderately correlated with the physician visual analogue scale scoring (r for rater 1 = 0.48, p=0.007; r for rater 2 = 0.36, p=0.0049). For the PVAS, scoring by rater 1 strongly correlated with the physician visual analogue scale (r = 0.50, p=0.004) whilst scoring by rater 2 moderately correlated (r = 0.37, p=0.043). Overall, the correlation of the visual analogue scale with both tools were very similar and the graphs are comparable in distribution (**Figure *7***).

Table 9 | Cohort characteristics of the randomly selected subgroup also scores with the visual analogue scale.

Parameter	Children scored with visual analogue scale (n = 30)	All children scored (n = 153)
Male, number (%)	15 (50)	83 (54)
Age, years, median (range)	5.6 (0.9-16.7)	5.7 (0.6-16.7)
Weight, kg, median (range)	20.3 (9.2-66.8)	20.7 (8.1-71.4)
Height, cm, median (range)	114.4 (65.0-181.1)	113.8 (65.0-181.1)
Blood pressure, mmHg, median (range)	116 (97-144)	117 (84-191)
Temperature, °C, median (range)	37.6 (35.3-39.3)	37.5 (35.3-39.9)
Serum creatinine, µmol/L, median (range)	36 (22-66)	40.0 (20.0-116.0)
Urine creatinine, mmol/L, median (range)	8.9 (1.3-30.9)	8.6 (0.9-30.9)
U-ACR, mg/mm Cr, median (range)	4.3 (0.5-94.3)	5.5 (0.5-3642.0)
U-PCR, mg/mmol, median (range)	8.0 (0.9-177.8)	10.3 (0.9-6866.5)
eGFR, mL/min/1.73m^2, median (range)	117.7 (64.8-161.7)	117.4 (53.3-213.2)
Platelet count, number (%)	21 (70)	119 (78)
Renal biopsy, number (%)	1 (3)	9 (6)
Skin biopsy, number (%)	2 (7)	15 (10)
Visual analogue scale score, median (range)	3 (1-10)	n/a

Figure 7 | The correlation of the visual analogue scale with the IgA-VAS for rater 1 (A) and rater 2 (B) (correlation coefficient for rater 1 = 0.48, *p*=0.007; for rater 2 = 0.36, *p*=0.0049) and with the PVAS for rater 1 (C) and rater 2 (D) (correlation coefficient for rater 1 = 0.50, *p*=0.004; for rater 2 = 0.37; *p*=0.043).



3.3.3 Concurrent validity

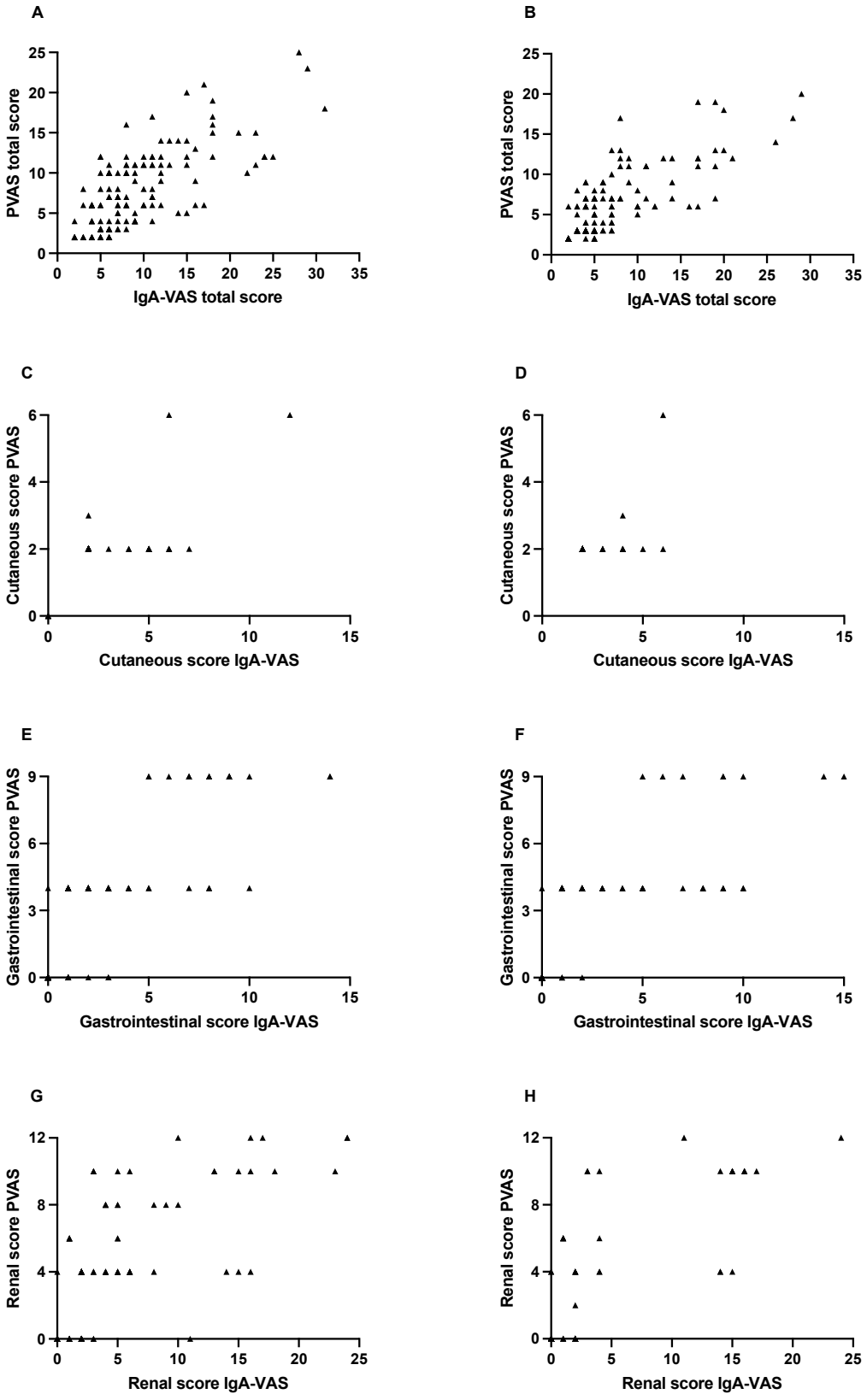
The overall number of children identified as having organ involvement using the PVAS has been described in **Table *10***. The median overall scores for the PVAS for rater 1 and rater 2 respectively were 6/63 (range 2-25) and 5/63 (range 2-20). For the subsystems, median scores for rater 1 and 2 respectively were 0/3 (range 0-3) and 1/3 (range 0-3) for general manifestations; 2/6 (range 0-6) and 2/6 (range 2-6) for cutaneous disease; 0/6 (range 0-2) and 0/6 range (0-2) for eye/mucous membrane symptoms; 0/6 (range 0-4) and 0/6 (range 0-0) for ENT manifestations; 0/6 (range 0-0) for both raters for chest and cardiovascular symptoms; 0/9 (range 0-9) for both raters for abdominal symptoms; 0/12 (range 0-12) for both raters for renal manifestations; and 0/9 (range 0-3) and 0/9 (0-0) for nervous system disease.

When directly analysing the overlapping domains between the IgA-VAS and the PVAS, there was a strong correlation, including the total score (all r>0.5, p<0.0001; **Figure 8**).

Table 10 | The number of children identified as having organ involvement using the PVAS.

Organ system	No. of patients (n = 153)	
	PVAS	IgA-VAS
Cutaneous	148	148
Gastrointestinal	60	69
Musculoskeletal	n/a	95
Renal	52	89
Other	n/a	35
General	58	n/a
Mucous membranes/eyes	1	n/a
ENT	0	n/a
Chest	0	n/a
Cardiovascular	0	n/a
Nervous system	0	n/a

Figure 8 | The correlation between overall scores for both the IgA-VAS and the PVAS for rater 1 (A; r=0.74) and rater 2 (B); r=0.78; both p<0.0001). A strong positive correlation was also found between the three overlapping subsystems for rater 1 and 2 respectively: cutaneous (C, r=0.64; D, r=0.54; both p<0.0001), gastrointestinal (E, r=0.86; F, r=0.80; both p<0.0001), and renal (G, r=0.75; H, r=0.83; both p<0.0001).



3.3.5 Inter-rater reliability

The overall inter-rater reliability for the total score of the IgA-VAS was unacceptable (0.13, $p<0.001$) and for the PVAS was poor (0.23, p<0.001**; Figure 9**). The IgA-VAS marginally outperformed the PVAS in the cutaneous domain (0.33 vs 0.21, both $p<0.001$) however both reliability coefficients were still poor. Inter-rater reliability for the gastrointestinal domains were both good (0.54 vs 0.58, both $p<0.001$) and for the musculoskeletal domain was very good for the IgA-VAS (0.67, $p<0.001$). Inter-rater reliability was poor for the renal domain (0.24 vs 0.30, both $p<0.001$). For the "other" domain in the IgA-VAS and the general subsystem in the PVAS, reliability was poor (0.29 vs 0.35 respectively, $p<0.001$, **Figure 10 and Figure 11**).

Figure 9 | The inter-rater agreement of the overall scores for the IgA-VAS (A, κ=0.13) and the PVAS (B, κ=0.23; both p<0.001).

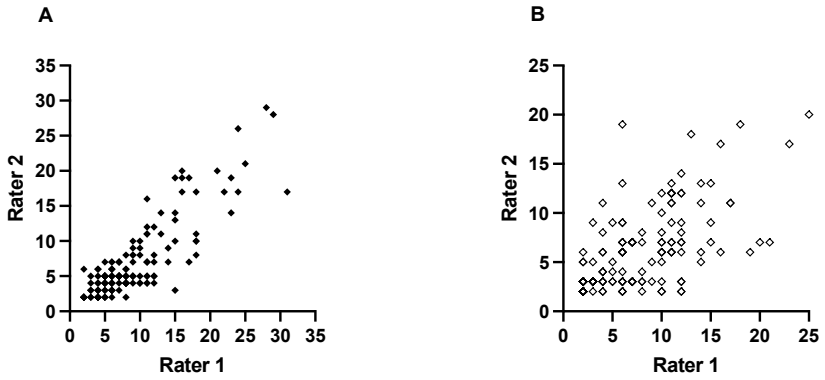


Figure 10 | The inter-rater reliability of the subdomains of the IgA-VAS: cutaneous (A, κ=0.33), gastrointestinal (B, κ=0.54), musculoskeletal (C, κ=0.67), renal (D, κ=0.24), and other (E, κ=0.29; all p<0.001).

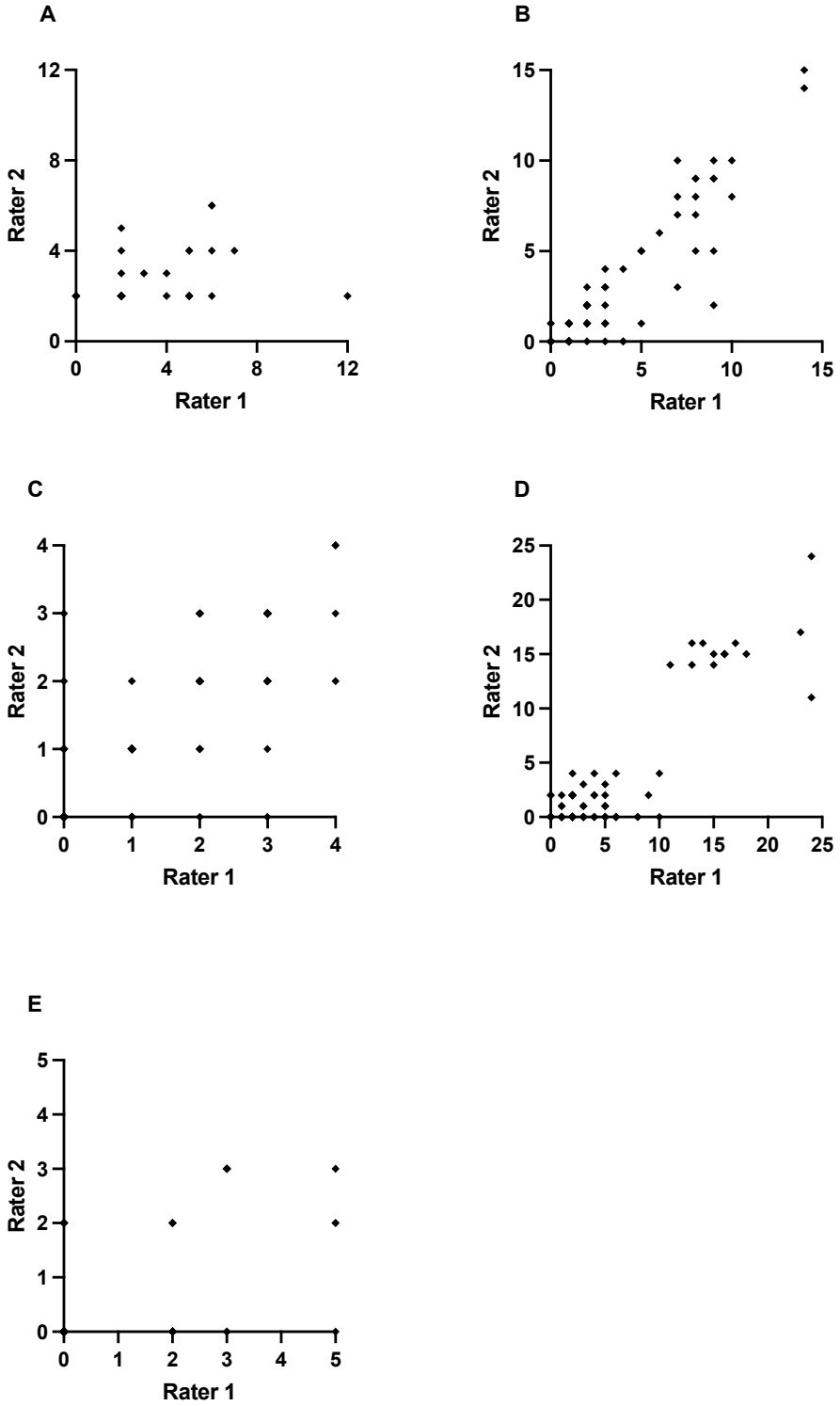
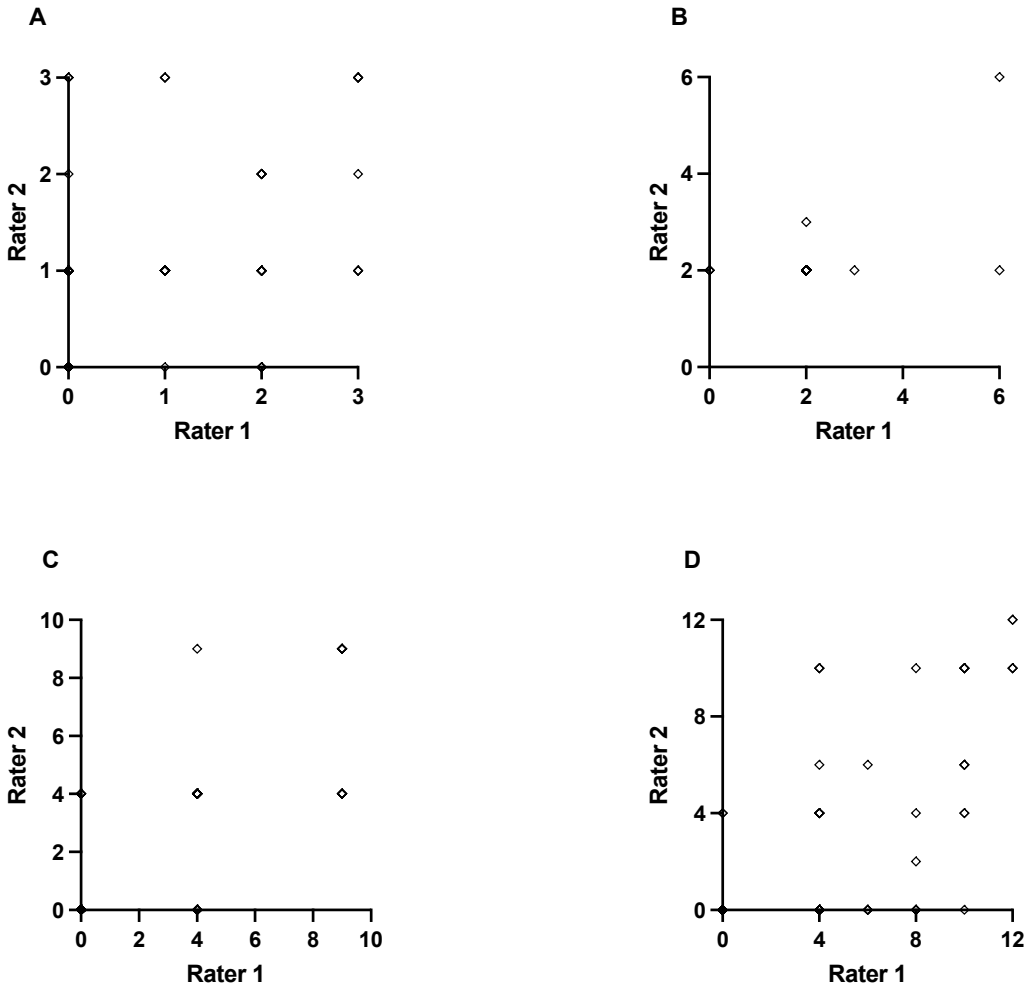


Figure 11 | The inter-rater reliability for the subsystems of the PVAS: general (A, κ=0.35), cutaneous (B, κ=0.21), abdominal (C, κ=0.58), and renal (D, 0.30; all p<0.001).



3.4 Discussion

Disease activity in any condition needs to be measured in order to make treatment decisions, objectively describe manifestations, assess prognosis, compare patients, and evaluate response to treatment over time. We have developed and preliminarily validated the IgA-VAS, a disease specific activity scoring tool, in a cohort of 153 paediatric patients with IgAV at a single centre. Content validity, construct validity, concurrent validity and inter-rater reliability were assessed as part of this preliminary validation study.

3.4.1 Validity and reliability

The IgA-VAS was designed to align with some principles of the PVAS, however, in terms of content validity, it appears that the IgA-VAS may be more suitable for use in IgAV. Feedback from the content validity study was incorporated into the tool before the retrospective scoring took place. The addition of further descriptions of rash distribution, pain, and renal involvement as well as the inclusion of rarer manifestations have further improved the disease-specific content validity of this tool. Although content or face validity was not performed for the PVAS in this chapter, no patients scored at all in four domains: ENT, cardiovascular, chest, and nervous system; the mucous membranes/eyes domain had one patient score for mouth ulcers. This supports the need for an alternative tool. Through the scoring process, we suggested some minor amendments to improve the tool including the order of manifestations to optimise ease of use i.e., whether they should be further grouped or put in order of weighting; further details regarding whether to score the worst symptom or all e.g., if a patient has gross haematuria, should they be scored just for gross haematuria or for both microscopic and gross haematuria; the addition of a section for endoscopy findings; and the consistency of terms used. Additionally, there may be a need for instructions on how to use and navigate the tool.

Construct validity was measured using a visual analogue scale and correlation with the IgA-VAS and the PVAS was reasonable. Controversy over the usefulness of a visual analogue scale in this setting exists, particularly as previous studies have suggested that they are largely influenced by external factors and is, in fact, a subjective rather than objective measure of disease activity (39, 94). A visual analogue scale is more useful in the validation of a scoring tool when there is no alternative gold standard for comparison therefore this was not essential for this chapter because the PVAS exists.

The PVAS is a well-established tool, considered the gold-standard for monitoring disease activity in childhood vasculitis, and has already undergone validation for a range of childhood vasculitidies. Despite the lack of patients with IgAV who were included in the PVAS validation study (40), we did observe a strong correlation between the two tools, particularly in the gastrointestinal domains,

therefore achieving the aim of aligning the design of the IgA-VAS with the design of the PVAS and assessing concurrent validity.

Both tools had a low overall inter-rater reliability, however the gastrointestinal domains in the IgA-VAS and the PVAS, as well the IgA-VAS musculoskeletal domain, demonstrated greater reliability. We observed a poor inter-rater reliability for the cutaneous and renal domains however this was consistent across both tools. Important reasons explaining this poor reliability are firstly due to the retrospective nature of this chapter leading to difficulties in finding relevant clinical information; secondly due to the large difference in the clinical experience of the two raters; and thirdly because of the inconsistency in reporting signs and symptoms at the time of diagnosis.

3.4.2 Domains of the IgA-VAS

Regarding the cutaneous manifestations of IgAV, the IgA-VAS was felt to capture the nature of the rash much more clearly than the PVAS. Most children only scored for "purpura" in the PVAS and there were few other relevant cutaneous descriptions other than the occasional patient who scored for gangrene or ulceration. The IgA-VAS, however, incorporated distribution of the rash which helped to build a bigger picture of describing disease activity.

The gastrointestinal section in the IgA-VAS was also felt to summarise abdominal involvement more clearly, which is suggested by the good inter-rater reliability and wider range of scores. The PVAS only includes two relevant abdominal manifestations, pain and GI bleeding, the IgA-VAS has a broader range of symptoms including different severities of pain, diarrhoea, and vomiting. However, during the scoring process it was apparent that some children with severe abdominal pain who underwent investigative imaging were not accounted for. Although intussusception was one of the criteria, there was no option to score for other endoscopy findings such as intramural bleeding.

Musculoskeletal manifestations were not included as their own domain in the PVAS and instead were grouped with general signs and symptoms. For patients with IgAV, therefore, it does not accurately capture joint disease as the total domain score would also include fever and weight loss. According to our data, the IgA-VAS identified 62.1% of patients with joint involvement compared to the PVAS which identified 24.1%. The literature suggests the rate of joint involvement to be 78.5-90% and therefore the PVAS may be vastly under-reporting musculoskeletal manifestations (9, 10). It also does not distinguish the severity of joint involvement, grouping both arthralgia and/or arthritis as one criterion.

Considering the renal domains, both tools were similar during the scoring process. The IgA-VAS included some extra manifestations such as microscopic vs gross haematuria, the severity of proteinuria, nephrotic syndrome, and histological nephritis. The addition of proteinuria severity and nephrotic syndrome are important as they are considered prognostic markers (1, 95).

The addition of the "other" domain was of importance, in particular for the identification of orchiditis, which was found to be present in 13.2% of male patients, a statistic similar to the literature and this is not a manifestation captured in the PVAS (9).

3.4.3 Limitations

There are some limitations that should be addressed. Firstly, the limitations associated with retrospective data. Both raters examined the medical records independently to find the information needed to score the patients, and therefore there were some discrepancies in the available data which was simply due to correctly identifying the relevant notes. Inevitably there was missing data that was unable to be accounted for. In some cases, patients presenting to Alder Hey had uncomplicated disease and therefore some investigations were not performed. This led to data assumptions, for example, for the heights and weights of some patients which was likely due to them presenting to accident and emergency (A&E) as, until recently, it was not standard practice to measure a child's height and weight in the emergency department. In this case, there would have been a knock-on effect to other results such as thresholds for normal blood pressure parameters and eGFR calculations, and these assumptions may have affected the accuracy of the results. Further, some investigations required to score patients using the PVAS, e.g., U-PCR and 24-hour urinary protein were not standard practice. Therefore, conversions from a U-ACR were used to estimate their equivalents, however it is possible that these conversions were inaccurate. A major limitation is the lack of standardisation in the reporting of patients with IgAV. The diagnosis and investigations performed were largely up to the discretion of the clinician who saw the patient. For example, there were often different descriptions of the same rash which made it difficult to interpret and therefore score. This further highlights the need for an objective measure of disease activity such as the IgA-VAS in partnership with national guidelines to standardise clinical care. Additionally, there was a large difference in the knowledge and clinical experience of both raters and this may have affected the scoring and interpretation of medical notes.

During the 5-year period in which patients were identified, there was a change from paper to online record keeping. This meant that some of the earlier diagnosed patients had less data available. Another change in policy occurred during this period regarding the diagnosis of IgAV. Whereas

children who had the characteristic rash were able to be diagnosed clinically with IgAV, the local guidelines were updated to align with EULAR/PRINTO/PReS classification so that children needed thrombocytopenia excluding before confirmation of IgAV (13). Therefore, not all patients had a platelet count to rule out idiopathic thrombocytopenic purpura (ITP) or thrombotic thrombocytopenic purpura (TTP) and it is possible that some patients may have been misclassified in the earlier time period. Additionally, Alder Hey is a tertiary paediatric referral centre in the North West of England and as such it is more likely to be referred complex cases for subspecialist care from a wide catchment of district general hospitals that include patients from North Wales, Stoke and Preston. It is more likely that this centre will have seen patients with more complex and severe disease and therefore the findings may not be generalisable to all cases of IgAV.

3.4.4 Further work

Although the IgA-VAS performed inadequately regarding inter-rater reliability, the tool has a high validity and therefore it is unlikely that the IgA-VAS will need to undergo further face, content, construct, and concurrent validity. However, further work and refinement is needed to optimise the tool before prospective validation. This should include a glossary or brief instructions on how to use the scoring tool and incorporating the changes suggested to the content. An updated version of the IgA-VAS has been developed based on the content validity (**Appendix *10***).

Prior to a prospective study, the revised IgA-VAS could undergo further face validation to confirm acceptance by resending it to original reviewers and perhaps extending the invitations to a wider audience that covers more specialities involved in the care of patients with IgAV. Following this, training case reports could be given to the future raters, similar to the PVAS validation study (40). This would involve a group of experts being given 20-30 written case reports of paediatric patients with IgAV for the raters to score independently, followed by a group discussion of the cases and a resolution resulting in a definitive score. Following this training, a cohort of patients should be scored independently by two raters on the same day to assess for inter-rater reliability as part of a prospective, multicentre validation study. Additionally, a longitudinal study could be implemented during the recommended follow up period of patients with IgAV to assess disease activity over time, response to treatment, and how these correlate with IgA-VAS scoring. As we already know that concurrent validity has been achieved, it may not be necessary to include PVAS scoring in a prospective study. Additionally, as the PVAS exists, it may not be necessary to include a comparison of a visual analogue scale in a prospective study as it would not be considered the gold standard method of measuring disease activity in IgAV, and we have already shown a strong positive correlation between the IgA-VAS and the visual analogue scale.

3.5 Conclusion

The IgA-VAS performed adequately in face, content, construct, and concurrent validation however further work is needed to optimise the tool before prospective validation to re-assess inter-rater reliability.

4. Discussion

The main body of this thesis centres around developing methods of measuring disease activity in IgA vasculitis. Specifically, this work considered reviewing the current evidence for urine biomarkers in detecting and assessing the severity of nephritis as a complication of IgAV, and the development and preliminary validation of a new scoring tool, the IgA-VAS, which was designed to be able to objectively measure and describe a child's disease activity.

One of the biggest issues facing children and their families after receiving a diagnosis of IgAV is the prospect and uncertainty surrounding the development of long-term sequalae i.e., IgAV-N. Currently, there are no established national guidelines to suggest how and for how long children should be monitored for renal involvement following their initial diagnosis. The general consensus is to follow children up for a 6-month period with blood pressure measurements and urine monitoring. Proteinuria and haematuria are reasonable markers of renal damage and may be present in up to 50% of children with IgAV, however they are unable to determine which patients will recover from nephritis spontaneously, which may need treatment, which may need a biopsy, and which will develop CKD. It is unreasonable to suggest performing a biopsy on every child with IgAV due to its invasive nature and risk of complications, and there may not be histological changes seen in the early stages of the disease. However, earlier detection is important as a guide to triage children in terms of risk and to provide information to support their families with the ultimate aim to allow identification of a suitable point to introduce nephritis-preventative treatment options. To do this, two things are required. Firstly, a better understanding of the pathophysiology of nephritis is required in order to develop new treatment options which may directly target the factors driving renal inflammation. Secondly, we need a better a way of objectively measuring IgAV disease activity that could be used as outcome measures for comparison pre- and post-intervention. Both urine biomarkers and a validated disease activity scoring tool would help to improve the outcomes of children with IgAV and increase the evidence base around the condition. In the future, it may be possible to incorporate a validated urine biomarker panel into the IgA-VAS, or alternatively the IgA-VAS may be used to standardise the reporting of the clinical characteristics in further studies evaluating urine biomarkers.

4.1 Limitations

There are limitations to this thesis which should be discussed. Due to time constraints, it was not possible to complete the full validation of the IgA-VAS or the laboratory work which should have followed on from chapter 2. However, this work has enhanced the evidence base surrounding disease activity monitoring in IgAV, and it will provide a foundation for future studies. Both chapter 2 and

chapter 3 highlighted the heterogeneity in reporting results during studies and those contained within medical notes. The limited ability to perform a meta-analysis further highlights the need for standardised reporting.

Another limitation is the patient group included in the chapters of this thesis. In chapter 3, we used data from a tertiary centre which receives more patients who are severely unwell with IgAV and is less likely to include patients with a simple disease course who may only present to primary or secondary paediatric care. Similarly in chapter 2 as many of the studies were small, there may have been skewed populations with more unwell patients being chosen for the biomarker assay. This may influence how representative the findings are to all patients with IgAV. Further, none of the studies in chapter 2 included an autoimmune control group which would've strengthened the data. Therefore, it is difficult to understand whether the biomarkers were specific to IgAV or whether they may have appeared in other renal inflammatory conditions.

4.2 Further work

This thesis has provided a good foundation for further study. Regarding urine biomarkers for IgAV-N, prospective longitudinal studies are needed with large biomarker panels and in-depth analysis which could include ROC curve analysis. Chapter 2 suggested that there are multiple potential biomarkers which could be used to identify nephritis or predict its severity and therefore it would be wise to focus future studies on more than one biomarker. As mentioned in the limitations, it would be pertinent to consider an autoimmune control group in further studies. Where possible, further studies should have large sample sizes and should be taken from multiple centres across the UK with later validation internationally.

Both chapter 2 and 3 have identified a need for standardised reporting of patients with IgAV which is easy to both use and interpret. It also highlighted that the PVAS is likely unsuitable for use, as it had a low inter-rater reliability and some key disease features were not detailed sufficiently. The IgA-VAS aimed to improve this and will need further prospective validation to determine its inter-rater reliability using the revised version. This should be done by at least two independent raters who have a similar level of experience and are adequately trained to use the tool and performed in a large cohort of paediatric patients in various settings, i.e., in primary, secondary, and tertiary care. This should eliminate some of the limitations that were identified in chapter 3 and the IgA-VAS may then be a suitable disease activity measure for clinical and research purposes.

5. Conclusion

To conclude, this thesis has considered the development of two methods of monitoring disease activity in IgA vasculitis, the most common vasculitis of childhood in the UK. This work has discovered a number of different biomarkers which have the potential to either identify or measure the severity of IgAV-N and highlight the potential importance of tubulointerstitial involvement in what was previously thought to be solely a glomerulonephritis. Further, we have created and developed a new scoring tool, the IgA-VAS, which has undergone preliminary retrospective validation and performed well in face, content, construct, and concurrent validity. Future studies should focus on multicentre prospective studies for biomarker discovery and validation of the IgA-VAS in a large cohort of paediatric patients.

6. References

1. 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.

2. Piram M, Mahr A. Epidemiology of immunoglobulin A vasculitis (Henoch–Schönlein): current state of knowledge. Current Opinion in Rheumatology. 2013;25(2).

3. 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;360(9341):1197-202.

4. 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. 2018;97(36):e12217-e.

5. Mizerska-Wasiak M, Gajewski Ł, Cichoń-Kawa K, Małdyk J, Dziedzic-Jankowska K, Leszczyńska B, et al. Serum GDIgA1 levels in children with IgA nephropathy and Henoch-Schönlein nephritis. Cent Eur J Immunol. 2018;43(2):162-7.

6. 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). Autoimmun Rev. 2017;16(12):1246-53.

7. Kiryluk K, Moldoveanu Z, Sanders JT, Eison TM, Suzuki H, Julian BA, et al. Aberrant glycosylation of IgA1 is inherited in both pediatric IgA nephropathy and Henoch-Schönlein purpura nephritis. Kidney international. 2011;80(1):79-87.

8. He X, Yu C, Zhao P, Ding Y, Liang X, Zhao Y, et al. The genetics of Henoch–Schönlein purpura: a systematic review and meta-analysis. Rheumatology International. 2013;33(6):1387-95.

9. Jauhola O, Ronkainen J, Koskimies O, Ala-Houhala M, Arikoski P, Hölttä T, et al. Clinical course of extrarenal symptoms in Henoch-Schonlein purpura: a 6-month prospective study. Arch Dis Child. 2010;95(11):871-6.

10. 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. 2001;80(5).

11. 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;40(4):371-6.

12. Ozen S, Marks SD, Brogan P, Groot N, de Graeff N, Avcin T, et al. European consensus-based recommendations for diagnosis and treatment of immunoglobulin A vasculitis-the SHARE initiative. Rheumatology (Oxford). 2019;58(9):1607-16.

13. Ozen S, Pistorio A, Iusan SM, Bakkaloglu A, Herlin T, Brik R, 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(5):798-806.

14. Trimarchi H, Barratt J, Cattran DC, Cook HT, Coppo R, Haas M, et al. Oxford Classification of IgA nephropathy 2016: an update from the IgA Nephropathy Classification Working Group. Kidney Int. 2017;91(5):1014-21.

15. Koskela M, Ylinen E, Ukonmaanaho EM, Autio-Harmainen H, Heikkilä P, Lohi J, et al. The ISKDC classification and a new semiquantitative classification for predicting outcomes of Henoch-Schönlein purpura nephritis. Pediatr Nephrol. 2017;32(7):1201-9.

16. 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;40(5):859-64.

17. Lai H-C. Henoch-Schönlein Purpura With Intussusception: A Case Report. Pediatrics & Neonatology. 2010;51(1):65-7.

18. Oni L, Sampath S. Childhood IgA Vasculitis (Henoch Schonlein Purpura)-Advances and Knowledge Gaps. Front Pediatr. 2019;7:257.

19. Hooper JE, Lee C, Hindley D. Case report: bullous Henoch–Schönlein purpura. Archives of Disease in Childhood. 2016;101(2):124.

20. Chen CB, Garlapati S, Lancaster JD, Zinn Z, Bacaj P, Patra KP. Bullous Henoch-Schönlein purpura in children. Cutis. 2015;96(4):248-52.

21. 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 Rep Pediatr. 2013;2013:680208.

22. Wang X, Zhu Y, Gao L, Wei S, Zhen Y, Ma Q. Henoch-Schönlein purpura with joint involvement: Analysis of 71 cases. Pediatr Rheumatol Online J. 2016;14(1):20.

23. Prathiba Rajalakshmi P, Srinivasan K. Gastrointestinal manifestations of Henoch-Schonlein purpura: A report of two cases. World J Radiol. 2015;7(3):66-9.

24. Glomerulonephritis KCPGf. KDIGO Clinical Practice Guideline for Glomerulonephritis. Official Journal of the International Society of Nephrology. 2012(2).

25. Chartapisak W, Opastirakul S, Hodson EM, Willis NS, Craig JC. Interventions for preventing and treating kidney disease in Henoch-Schönlein Purpura (HSP). Cochrane Database Syst Rev. 2009(3):Cd005128.

26. 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(8):Cd005128.
27. Koç MÖ, Dursun H, Kural B, Hatipoğlu S. Organ involvement in immunoglobulin a vasculitis (Henoch-Shönlein purpura) children: Relation to immune profile. The Egyptian Rheumatologist. 2020;42(3):219-23.
28. Hong J, Yang HR. Laboratory markers indicating gastrointestinal involvement of henoch-schönlein purpura in children. Pediatr Gastroenterol Hepatol Nutr. 2015;18(1):39-47.
29. Shen R, Ren X, Jing R, Shen X, Chen J, Ju S, et al. Rheumatoid Factor, Anti-Cyclic Citrullinated Peptide Antibody, C-Reactive Protein, and Erythrocyte Sedimentation Rate for the Clinical Diagnosis of Rheumatoid Arthritis. Lab Med. 2015;46(3):226-9.
30. Saulsbury FT, Kirkpatrick PR, Bolton WK. IgA antineutrophil cytoplasmic antibody in Henoch-Schönlein purpura. Am J Nephrol. 1991;11(4):295-300.
31. Torraca PF, Castro BC, Hans GF. Henoch-Schönlein purpura with c-ANCA antibody in an adult. An Bras Dermatol. 2016;91(5):667-9.
32. Strimbu K, Tavel JA. What are biomarkers? Curr Opin HIV AIDS. 2010;5(6):463-6.
33. TESCH GH. Review: Serum and urine biomarkers of kidney disease: A pathophysiological perspective. Nephrology. 2010;15(6):609-16.
34. Califf RM. Biomarker definitions and their applications. Exp Biol Med (Maywood). 2018;243(3):213-21.
35. Huss R. Chapter 19 - Biomarkers. In: Atala A, Allickson JG, editors. Translational Regenerative Medicine. Boston: Academic Press; 2015. p. 235-41.
36. Wells G, Becker JC, Teng J, Dougados M, Schiff M, Smolen J, et al. Validation of the 28-joint Disease Activity Score (DAS28) and European League Against Rheumatism response criteria based on C-reactive protein against disease progression in patients with rheumatoid arthritis, and comparison with the DAS28 based on erythrocyte sedimentation rate. Ann Rheum Dis. 2009;68(6):954-60.
37. Mease PJ. Measures of psoriatic arthritis: Tender and Swollen Joint Assessment, Psoriasis Area and Severity Index (PASI), Nail Psoriasis Severity Index (NAPSI), Modified Nail Psoriasis Severity Index (mNAPSI), Mander/Newcastle Enthesitis Index (MEI), Leeds Enthesitis Index (LEI), Spondyloarthritis Research Consortium of Canada (SPARCC), Maastricht Ankylosing Spondylitis Enthesis Score (MASES), Leeds Dactylitis Index (LDI), Patient Global for Psoriatic Arthritis, Dermatology Life Quality Index (DLQI), Psoriatic Arthritis Quality of Life (PsAQOL), Functional Assessment of Chronic Illness Therapy–Fatigue (FACIT-F), Psoriatic Arthritis Response Criteria (PsARC), Psoriatic Arthritis Joint Activity Index (PsAJAI), Disease Activity in Psoriatic Arthritis (DAPSA),

and Composite Psoriatic Disease Activity Index (CPDAI). Arthritis Care & Research. 2011;63(S11):S64-S85.

38. Jesus D, Matos A, Henriques C, Zen M, Larosa M, Iaccarino L, et al. Derivation and validation of the SLE Disease Activity Score (SLE-DAS): a new SLE continuous measure with high sensitivity for changes in disease activity. Ann Rheum Dis. 2019;78(3):365-71.

39. Luqmani RA, Bacon PA, Moots RJ, Janssen BA, Pall A, Emery P, et al. Birmingham Vasculitis Activity Score (BVAS) in systemic necrotizing vasculitis. Qjm. 1994;87(11):671-8.

40. Dolezalova P, Price-Kuehne FE, Özen S, Benseler SM, Cabral DA, Anton J, et al. Disease activity assessment in childhood vasculitis: development and preliminary validation of the Paediatric Vasculitis Activity Score (PVAS). Annals of the Rheumatic Diseases. 2013;72(10):1628.

41. Mukhtyar C, Lee R, Brown D, Carruthers D, Dasgupta B, Dubey S, et al. Modification and validation of the Birmingham Vasculitis Activity Score (version 3). Ann Rheum Dis. 2009;68(12):1827-32.

42. 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(9):916-20.

43. Huang X, Ma L, Ren P, Wang H, Chen L, Han H, et al. Updated Oxford classification and the international study of kidney disease in children classification: application in predicting outcome of Henoch-Schönlein purpura nephritis. Diagn Pathol. 2019;14(1):40.

44. Downes MJ, Brennan ML, Williams HC, Dean RS. Development of a critical appraisal tool to assess the quality of cross-sectional studies (AXIS). BMJ Open. 2016;6(12):e011458.

45. Mandrekar JN. Receiver Operating Characteristic Curve in Diagnostic Test Assessment. J Thorac Oncol. 2010;5(9):1315-6.

46. An JK, Xia D. Diagnostic performance of urinary proteins as biomarkers in evaluating Henoch Schonlein purpura nephritis. Clin Exp Med. 2018;11(11):12354-60.

47. Fuentes Y, Hernández AM, García-Roca P, Valverde S, Velásquez-Jones LF, Sosa G, et al. Urinary MCP-1/creatinine in Henoch-Schönlein purpura and its relationship with nephritis. Pediatr Nephrol. 2014;29(6):1047-52.

48. 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.

49. Fang X, Wu HY, Lu M, Cao Y, Wang R, Wang MQ, et al. Urinary proteomics of Henoch-Schonlein purpura nephritis in children using liquid chromatography-tandem mass spectrometry. Clinical Proteomics. 2020;17(1).

50. Ma YF, Li YF, Guo GM, Zhu YJ, Gong YL, Dong Y. Changes of new urinary biomarkers in children with Henoch-Schonlein purpura nephritis. J Shanghai Jiaotong Univ Med Sci. 2020;40(6):842-6.
51. Qin YH, Zhou TB, Lei FY, Huang WF, Zhao YJ, Lin FQ, et al. Cut-off values for serum matrix metalloproteinase-9: is there a threshold to predict renal involvement for Henoch-Schonlein purpura in children? J Nephrol. 2011;16(1):93-9.
52. Ge W, Wang HL, Sun RP. Pentraxin 3 as a novel early biomarker for the prediction of Henoch-Schönlein purpura nephritis in children. Eur J Pediatr. 2014;173(2):213-8.
53. Mao YN, Liu W, Li YG, Jia GC, Zhang Z, Guan YJ, et al. Urinary angiotensinogen levels in relation to renal involvement of Henoch-Schonlein purpura in children. J Nephrol. 2012;17(1):53-7.
54. Wang JP, Li YY, Chen YL, Dai XH, Di YZ, Shen MJ, et al. Urinary Macrophage Migration Inhibitory Factor as a Noninvasive Biomarker in Pediatric Henoch-Schonlein Purpura Nephritis. J Clin Rheumatol. 2017;23(5):258-61.
55. Zhang J, Zeng H, Wang N, Tian X, Dou W, Shi P. Beneficial effects of creatine phosphate sodium for the treatment of Henoch-Schönlein purpura in patients with early renal damage detected using urinary kidney injury molecule-1 levels. Eur J Pediatr. 2015;175(1):49-55.
56. Pillebout E, Jamin A, Ayari H, Housset P, Pierre M, Sauvaget V, et al. Biomarkers of IgA vasculitis nephritis in children. PLoS One. 2017;12(11):e0188718.
57. Ye Q, Shang SQ, Liu AM, Zhang T, Shen HQ, Chen XJ, et al. 24h Urinary Protein Levels and Urine Protein/Creatinine Ratios Could Probably Forecast the Pathological Classification of HSPN. PLoS One. 2015;10(5):e0127767.
58. Wang J, Ying Q, Zhong S, Chen Y, Di Y, Dai X, et al. Elevated urinary monocyte chemoattractant protein-1 levels in children with Henoch-Schonlein purpura nephritis. Pediatr Neonatol. 2017;59(3):238-43.
59. Wang J, Li Y, Chen Y, Dai X, Di Y, Shen M, et al. Urinary Macrophage Migration Inhibitory Factor as a Noninvasive Biomarker in Pediatric Henoch-Schonlein Purpura Nephritis. J Clin Rheumatol. 2017;23(5):258-61.
60. Ge W, Wang H-L, Sun R-P. Pentraxin 3 as a novel early biomarker for the prediction of Henoch-Schonlein purpura nephritis in children. European journal of pediatrics. 2014;173(2):213-8.
61. Qin Y-H, Zhou T-B, Lei F-Y, Huang W-F, Zhao Y-J, Lin F-Q, et al. Cut-off values for serum matrix metalloproteinase-9: is there a threshold to predict renal involvement for Henoch-Schonlein purpura in children? Nephrology (Carlton, Vic). 2011;16(1):93-9.

62. Wang J, Ying Q, Zhong S, Chen Y, Di Y, Dai X, et al. Elevated urinary monocyte chemoattractant protein-1 levels in children with Henoch-Schonlein purpura nephritis. Pediatr Neonatol. 2018;59(3):238-43.
63. Song J, Yu J, Prayogo GW, Cao W, Wu Y, Jia Z, et al. Understanding kidney injury molecule 1: a novel immune factor in kidney pathophysiology. Am J Transl Res. 2019;11(3):1219-29.
64. Han WK, Bailly V, Abichandani R, Thadhani R, Bonventre JV. Kidney Injury Molecule-1 (KIM-1): A novel biomarker for human renal proximal tubule injury. Kidney International. 2002;62(1):237-44.
65. Edelstein CL. Chapter Six - Biomarkers in Acute Kidney Injury. In: Edelstein CL, editor. Biomarkers of Kidney Disease (Second Edition): Academic Press; 2017. p. 241-315.
66. Waanders F, van Timmeren MM, Stegeman CA, Bakker SJL, van Goor H. Kidney injury molecule-1 in renal disease. J Pathol. 2010;220(1):7-16.
67. Zhang Y, Li A, Wen J, Zhen J, Hao Q, Zhang Y, et al. Kidney Injury Molecule-1 Level is Associated with the Severity of Renal Interstitial Injury and Prognosis in Adult Henoch-Schönlein Purpura Nephritis. Arch Med Res. 2017;48(5):449-58.
68. Xu P-C, Zhang J-J, Chen M, Lv J-C, Liu G, Zou W-Z, et al. Urinary kidney injury molecule-1 in patients with IgA nephropathy is closely associated with disease severity. Nephrol Dial Transplant. 2011;26(10):3229-36.
69. Zhang J, Zeng H, Wang N, Tian X, Dou W, Shi P. Beneficial effects of creatine phosphate sodium for the treatment of Henoch-Schönlein purpura in patients with early renal damage detected using urinary kidney injury molecule-1 levels. Eur J Pediatr. 2016;175(1):49-55.
70. Liu F, Wang C, Wang R, Wang W, Li M. Henoch-schonlein Purpura Nephritis with Renal Interstitial Lesions. Open Med (Wars). 2018;13:597-604.
71. Deshmane SL, Kremlev S, Amini S, Sawaya BE. Monocyte chemoattractant protein-1 (MCP-1): an overview. J Interferon Cytokine Res. 2009;29(6):313-26.
72. Saitoh A, Suzuki Y, Takeda M, Kubota K, Itoh K, Tomino Y. Urinary levels of monocyte chemoattractant protein (MCP)-1 and disease activity in patients with IgA nephropathy. J Clin Lab Anal. 1998;12(1):1-5.
73. Wen X, Kellum JA. N-Acetyl-beta-D-Glucosaminidase (NAG). In: Vincent J-L, Hall JB, editors. Intensive Care Med. Berlin, Heidelberg: Springer Berlin Heidelberg; 2012. p. 1509-10.
74. Vaidya VS, Ferguson MA, Bonventre JV. Biomarkers of acute kidney injury. Annu Rev Pharmacol Toxicol. 2008;48:463-93.
75. Sheira G, Noreldin N, Tamer A, Saad M. Urinary biomarker N-acetyl-β-D-glucosaminidase can predict severity of renal damage in diabetic nephropathy. Diabetes Metab Syndr. 2015;14:4-.

76. Bazzi C, Petrini C, Rizza V, Arrigo G, Napodano P, Paparella M, et al. Urinary N-acetyl-beta-glucosaminidase excretion is a marker of tubular cell dysfunction and a predictor of outcome in primary glomerulonephritis. Nephrol Dial Transplant. 2002;17(11):1890-6.

77. Hosmer DW, Lemeshow S, Sturdivant RX, Hosmer DW, Jr. Applied Logistic Regression. New York, UNITED STATES: John Wiley & Sons, Incorporated; 2013.

78. Lantelme P, Rohrwasser A, Gociman B, Hillas E, Cheng T, Petty G, et al. Effects of dietary sodium and genetic background on angiotensinogen and Renin in mouse. Hypertension. 2002;39(5):1007-14.

79. Kobori H, Navar LG. Urinary Angiotensinogen as a Novel Biomarker of Intrarenal Renin-Angiotensin System in Chronic Kidney Disease. Int Rev Thromb. 2011;6(2):108-16.

80. Yoshioka T, Xu YX, Yoshida H, Shiraga H, Muraki T, Ito K. Deletion polymorphism of the angiotensin converting enzyme gene predicts persistent proteinuria in Henoch-Schönlein purpura nephritis. Arch Dis Child. 1998;79(5):394-9.

81. The SONG Initiative: Standardised Outcomes in Nephrology (SONG); [Available from: https://songinitiative.org/news/.

82. Kawasaki Y, Suyama K, Yugeta E, Katayose M, Suzuki S, Sakuma H, et al. The incidence and severity of Henoch-Schönlein purpura nephritis over a 22-year period in Fukushima Prefecture, Japan. Int Urol Nephrol. 2010;42(4):1023-9.

83. Middleton F. Reliability vs validity: what's the difference? : Scribbr; 2019 [Available from: https://www.scribbr.com/methodology/reliability-vs-validity/.

84. Middleton F. The four types of validity 2019 [Available from: https://www.scribbr.com/methodology/types-of-validity/.

85. Shantikumar S. Validity, reliability and generalisability: HealthKnoweldge; 2018 [Available from: https://www.healthknowledge.org.uk/content/validity-reliability-and-generalisability.

86. McLeod S. What is Validity? : SimplyPsychology; 2013 [Available from: https://www.simplypsychology.org/validity.html.

87. Brown JD. What is construct validity? 2000 [Available from: https://hosted.jalt.org/test/bro_8.htm.

88. The fourth report on the diagnosis, evaluation, and treatment of high blood pressure in children and adolescents. Pediatrics. 2004;114(2 Suppl 4th Report):555-76.

89. Counahan R, Chantler C, Ghazali S, Kirkwood B, Rose F, Barratt TM. Estimation of glomerular filtration rate from plasma creatinine concentration in children. Arch Dis Child. 1976;51(11):875-8.

90. Sumida K, Nadkarni GN, Grams ME, Sang Y, Ballew SH, Coresh J, et al. Conversion of Urine Protein–Creatinine Ratio or Urine Dipstick Protein to Urine Albumin–Creatinine Ratio for Use in Chronic Kidney Disease Screening and Prognosis. Annals of Internal Medicine. 2020;173(6):426-35.
91. Kamińska J, Dymicka-Piekarska V, Tomaszewska J, Matowicka-Karna J, Koper-Lenkiewicz OM. Diagnostic utility of protein to creatinine ratio (P/C ratio) in spot urine sample within routine clinical practice. Crit Rev Clin Lab Sci. 2020;57(5):345-64.
92. Random Number Generator: CalculatorSoup; [Available from: https://www.calculatorsoup.com/calculators/statistics/random-number-generator.php.
93. Helena Chmura Kraemer, Ph.D. ,, David J. Kupfer, M.D. ,, Diana E. Clarke, Ph.D. ,, William E. Narrow, M.D., M.P.H. , and, Darrel A. Regier, M.D., M.P.H. DSM-5: How Reliable Is Reliable Enough? American Journal of Psychiatry. 2012;169(1):13-5.
94. Demirkaya E, Ozen S, Pistorio A, Galasso R, Ravelli A, Hasija R, et al. Performance of Birmingham Vasculitis Activity Score and disease extent index in childhood vasculitides. Clin Exp Rheumatol. 2012;30(1 Suppl 70):S162-8.
95. Müller D, Greve D, Eggert P. Early tubular proteinuria and the development of nephritis in Henoch-Schönlein purpura. Pediatr Nephrol. 2000;15(1-2):85-9.

7. Appendices

Appendix 1 | The published version of chapter 2: A systematic review of urine biomarkers in children with IgA vasculitis nephritis.

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SYSTEMATIC REVIEW/META-ANALYSIS

A systematic review of urine biomarkers in children with IgA vasculitis nephritis

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Abstract

Background Nephritis is a recognised complication of IgA vasculitis (IgAV, Henoch-Schönlein purpura) contributing to 1–2% of all chronic kidney disease (CKD) stage 5. Improved understanding may reduce irreversible damage in IgAV nephritis (IgAV-N).

Objective The aim of this study was to perform a comprehensive systematic literature review to identify promising clinical and pre-clinical urine biomarkers in children with IgAV-N that could predict the presence of nephritis and/or determine its severity.

Methods A systematic literature review was performed using four search engines and a predefined search term strategy. Promising biomarkers were divided in terms of clinical or pre-clinical and ability to predict the presence of nephritis or determine its severity. Results were described using statistical significance ($p < 0.05$) and area under the curve (AUC) values.

Results One hundred twenty-one studies were identified; 13 were eligible. A total of 2446 paediatric patients were included: healthy controls ($n = 761$), children with IgAV-N ($n = 1236$) and children with IgAV without nephritis (IgAV-noN, $n = 449$). Fifty-one percent were male, median age 7.9 years. The clinical markers, 24-h protein quantity and urine protein:creatinine ratio, were deemed acceptable for assessing severity of nephritis (AUC < 0.8). Urinary albumin concentration (Malb) performed well (AUC 0.81–0.98). The most promising pre-clinical urinary biomarkers in predicting presence of nephritis were as follows: kidney injury molecule-1 (KIM-1) (AUC 0.93), monocyte chemotactic protein-1 (MCP-1) (AUC 0.83), N-acetyl-β-glucosaminidase (NAG) (0.76–0.96), and angiotensinogen (AGT) (AUC not available). Urinary KIM-1, MCP-1, and NAG appeared to correlate with disease severity.

Conclusions Longitudinal studies are needed to assess whether pre-clinical biomarkers enhance standard of care in IgAV-N.

Keywords IgA vasculitis · Henoch-Schönlein purpura · Nephritis · Children · Urine · Biomarker

Introduction

Immunoglobulin A (IgA) vasculitis (IgAV), formerly known as Henoch-Schönlein purpura (HSP), is the most common form of vasculitis in children, with an estimated incidence of 20.4 cases/100,000 childhood population [1, 2]. This systemic small vessel vasculitis usually presents with a palpable purpuric rash, plus polyarthritis, gastrointestinal (GI) symptoms and/or kidney involvement, and it is predominantly a disease of childhood. The exact pathophysiology is still unknown, but due to the high levels of galactose deficient IgA1 levels seen in IgAV patients, it is thought that aberrant IgA glycosylation is a contributor to the mechanism of disease. Immune complexes containing IgA1 then deposit in the small vessels activating an immune response and subsequent inflammation [3]. The prognosis of IgAV is usually excellent with 94% of children achieving full, spontaneous recovery within 2 years [4]. Around 40–50% of patients experience kidney inflammation (termed IgAV nephritis; IgAV-N) ranging from microscopic haematuria to rapidly progressive glomerulonephritis [5, 6] and it currently contributes to 1–2% of all chronic kidney disease (CKD) stage 5 [7]. For this reason, all patients should have a period of follow-up to screen for IgAV-N that currently consists of 6 months of periodic urinalysis and blood pressure monitoring, as surrogate clinical

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markers of kidney injury [8]. Identifying those individuals at greatest risk of kidney inflammation is believed to be the key to reducing the incidence of irreversible kidney damage in IgAV-N and allowing a personalised approach to monitoring. Pre-clinical biomarkers may have a role in identifying patients with or without nephritis and determining the severity of kidney inflammation. Ideally, to fulfil this role they should be reflective of the pathogenic biological process and be accurate and reproducible. For IgAV-N, this may provide earlier diagnosis of kidney inflammation, prognostic information, and scientific insight and ultimately allow personalised disease monitoring to stratify the management of children with this disease.

The primary aim of this study was to perform a comprehensive systematic literature review to identify promising clinical and pre-clinical urine biomarkers in children with IgAV that can either predict the presence of nephritis and/or determine its severity.

Methods

Study population

The inclusion criteria were paediatric participants (<18 years) of any sex and ethnicity, with a diagnosis of IgAV-N. A diagnosis of IgAV-N included any of the following: abnormal urinalysis; haematuria and/or a high urinary protein concentration within 6 months of the onset of rash; and/or a reduced estimated glomerular filtration rate (eGFR) in participants who had met the clinical diagnosis of IgAV [9]. The exclusion criteria were studies that involved adult participants (>18 years) or participants who had other forms of nephritis or vasculitis.

Intervention

The intervention of interest was biomarker assay evaluation in a urine sample.

Comparator

The study aimed to compare: (i) urine biomarkers that may determine the presence of nephritis in children with IgAV-N compared to children with IgAV and no nephritis (IgAV-noN) and/or healthy paediatric controls and (ii) urine biomarkers that may determine the severity of nephritis in children with IgAV-N.

Outcome

The outcome of interest was the identification of clinical or pre-clinical biomarkers that are able to determine the presence of nephritis as defined by each individual study and/or the severity defined in terms of the International Study of Kidney Disease in Children (ISKDC) classification histological grade or extent of proteinuria [10].

Study design

Data extraction

Using predefined methodology, this systematic review evaluated the current available literature. Four online databases, PubMed, Web of Science, Medline, and Scopus, were used with the following terms: (((((((((neonat*) OR (adolescen*)) OR (infan*)) OR (child*)) OR (pediatric*)) OR (paediatric*)) AND (((((((immunoglobulin A vasculitis) OR (IgA Vasculitis)) OR (IgAV)) OR (Henoch Sch*nlein purpura)) OR (Henoch-Sch*nlein purpura)) OR (HSP))) AND (((((((nephritis) OR (renal injur*)) OR (kidney injur*)) OR (renal damage*)) OR (kidney damage)) OR (ckd)) OR (chronic kidney disease))) AND (urin*)) AND (biomarker*). The studies included were meta-analyses, randomised control trials (RCTs), cohort studies, case-control studies, cross-sectional studies and case series ($n > 5$) that were all accessible in full text through the University of Liverpool, with at least an English abstract. Secondary data and animal studies were excluded, as well as papers with an original publication date before October 2000, allowing for a 20-year inclusion period. The reference lists of relevant literature were hand-searched to identify any additional eligible studies.

Data collection

From each included study, information was extracted on author, year of publication, study design, study population, definition of nephritis, type of sampling and laboratory technique, biomarkers assessed, and key findings. The relevant data was collected on a predesigned pro forma by the primary author (CW). Where full English transcripts were unavailable, data was extracted from the English abstract.

Quality appraisal and statistical analysis

The "Appraisal tool for Cross-Sectional Studies" (AXIS) tool was used, which comprises 20 questions to appraise and compare the quality of the literature [11]. Pre-clinical biomarkers identified in more than one paper were to be discussed in more detail. Those that have only been reported once were to be summarised in a data table (Table 1). The results will be described in terms of clinical or pre-clinical biomarkers. A clinical biomarker is defined as any biological marker that is available in a routine clinical laboratory. A pre-clinical biomarker is one that is not routinely available in a clinical laboratory and deemed experimental [25]. Where available,

Table 1 A table describing the data in each paper included in the systematic review

Author	Year	Study design	Cohort demographic	Definition of nephritis	Type of sampling	Laboratory technique	Biomarker	Results
An and Xia [12]	2018	Retrospective cross sectional	45 children with biopsy-confirmed IgAV-N grouped by pathological grade.	Kidney histology, classified according to ISKDC.	24-h urine collection	Turbidimetric method	Beta-2 microglobulin (β2-MG) Urinary albumin concentration (Malb) N-Acetyl-beta-glucosaminidase (NAG) Transferrin (TfR)	Malb, TfR and NAG were different according to pathological grades ($p < 0.05$). β2-MG was not statistically significantly increased.
Dyga et al. [13]	2020	Prospective longitudinal	11 paediatric patients IgAV-N (M = 10, F = 1) and 18 with IgAV-noN (M = 7, F = 11) compared to 34 healthy controls (M = 23, F = 11).	Haematuria: >5 erythrocytes per high power field ± UP/UC ratio > 30 mg/mmol ± eGFR < 60 mL/min/1.73 m^2.	One acute random urine sample and follow-up sample 2–6 months after discharge	ELISA	Neutrophil gelatinase-associated lipocalin (NGAL) Kidney injury molecule-1 (KIM-1) Liver-fatty acid binding protein (L-FABP)	Acutely, all three biomarkers were increased in children with IgAV compared to controls ($p < 0.001$), however, not between the IgAV-N and IgAV-noN groups. At follow-up, NGAL was found to be increased in IgAV-N compared to IgAV-noN ($p = 0.063$).
Fang et al. [14]	2020	Prospective cross sectional	30 children with IgAV-N (M = 20, F = 10) compared to 10 IgAV-noN (M = 6, F = 4) and 29 healthy controls (M = 12, F = 17).	Haematuria and/or high urinary protein concentration or kidney biopsy results showing mesangial IgA deposition.	Midstream morning urine sample	ELISA	Integrin beta-1 (ITGB1) Tenascin	There were decreased urinary concentrations of both biomarkers in the IgAV-N cohort compared to controls ($p < 0.05$). Tenascin was statistically significantly different in the IgAV-N vs. IgAV-noN ($p = 0.005$).
Fuentes et al. [15]	2014	Prospective cross sectional	57 children had IgAV-N (M = 32, F = 25) and 20 with IgAV-noN (M = 12, F = 8), compared to 25 healthy volunteers (M = 16, F = 9).	Haematuria (>5 cells per high-power field in urine sediment) and/or high urinary protein concentration. Kidney biopsy was classified using the ISKDC criteria.	First-morning urine sample	ELISA	Monocyte chemoattractant protein-1 (MCP-1)	Urinary MCP-1/Cr was increased in IgAV-N compared to the IgAV-noN and the controls ($p < 0.0001$).
Ge et al. [16]	2014	Prospective longitudinal	34 paediatric patients with IgAV-noN (M = 15, F = 18), 37 with IgAV-N (M = 18, F = 19) and 37 healthy children (M = 19, F = 18).	Haematuria and/or high urinary protein concentration.	24-h urine collection	ELISA	Urinary albumin concentration (Malb) Beta-2 microglobulin (β2-MG)	The concentrations were increased in IgAV-N patients compared to controls ($p < 0.05$) and IgAV-noN ($p < 0.05$).
Ma et al. [17]	2020	Prospective longitudinal	14 children with IgAV-N (M = 7, F = 7) vs. 28 with IgAV-noN (M = 16, F = 12) and 23 healthy volunteers (M = 9, F = 14).	N/A[a]	Morning urine sample	N/A[a]	Urinary angiotensinogen (UAGT) Fibroblast specific protein-1 (FSP-1) Thrombin	UAGT and FSP-1 were increased in the IgAV-N cohort compared to controls and IgAV-noN ($p < 0.05$). Thrombin was increased in all IgAV patients when compared to controls ($p < 0.05$).
	2012					ELISA		

Table 1 (continued)

Author	Year	Study design	Cohort demographic	Definition of nephritis	Type of sampling	Laboratory technique	Biomarker	Results
Mao et al. [18]		Prospective longitudinal	51 paediatric patients with IgAV-noN (M = 24, F = 27) compared to 43 with haematuria but a urinary protein concentration of 0 (M = 21, F = 22) and 13 with high urinary protein concentration (M = 5, F = 8).	Urinary protein concentration (>1.0 g/24 h) and/or haematuria.	24-h urine sample collected acutely and at follow-up		Urinary angiotensinogen (UAGT)	Acutely, UAGT concentrations were higher in those with a higher urinary protein concentration compared to IgAV-noN and IgAV with haematuria groups ($p < 0.0001$). During the convalescent phase, UAGT concentrations were increased in the patients with high urinary protein concentration compared to IgAV-noN patients ($p < 0.0001$) and the haematuria group ($p < 0.001$).
Pillebout et al. [19]	2017	Prospective cross sectional	21 paediatric controls (M = 13, F = 8) were compared to 17 children with IgAV-noN (M = 12, F = 5) and 33 children with IgAV-N (M = 20, F = 13).	The presence of haematuria and/or a PCR > 0.5 g/g and/or an eGFR < 60 mL/min/1.73 m^2.	N/A[b]	ELISA	IgA/Cr ratio (IgA/Cr) IgG/Cr ratio (IgG/Cr) IgM/Cr ratio (IgM/Cr) Igλ/IgK ratio (Igλ/IgK) IL-6/Cr ratio (IL-6/Cr) IL-8/Cr ratio (IL-8/Cr) IL-10/Cr ratio (IL10/Cr)	IgA/Cr and IgM/Cr were raised in IgAV-N compared to both controls and IgAV-noN ($p < 0.0001$). IgG/Cr and the Igλ/IgK ratios were increased in IgAV-N compared to IgAV-noN ($p < 0.01$). IL-6/Cr and IL-8/Cr were increased in IgAV-N compared to controls ($p < 0.0001$) and IgAV-noN ($p < 0.01$). IL-2/Cr was increased only when compared to IgAV-noN ($p < 0.01$).
Qin et al. [20]	2011	Prospective cross sectional	68 children with IgAV-noN (M = 33, F = 35) were compared to 66 with IgAV-N (M = 32, F = 34) and 60 controls (M = 29, F = 31).	Patients categorised into normal concentrations of protein and haematuria; low-grade urinary protein concentration (< 1 g/L) and/or haematuria; and high urinary protein concentration (≥1 g/L) and/or haematuria.	Mid-stream urine sample	ELISA	Matrix metalloproteinase-9 (MMP-9) Tissue inhibitor matrix metalloproteinase-1 (TIMP-1)	Urinary MMP-9, TIMP-1 and MMP-9/TIMP-1 were increased in IgAV-N compared to IgAV-noN ($p < 0.05$) and controls ($p < 0.01$). MMP-9 and MMP-9/TIMP-1 were increased in children with high urinary protein concentration compared to mild ($p < 0.01$) and moderate ($p < 0.05$).
Wang et al. [21]	2017	Prospective cross sectional	126 paediatric patients with IgAV-N (M = 66, F = 60) were compared to 135 non-nephritis IgAV children	Haematuria and/or high urinary protein concentration within 6 months of the onset of rash. IgAV-N patients were further	First-morning urine sample	ELISA	Monocyte chemoattractant protein-1 (MCP-1)	Urinary MCP-1 was increased in IgAV-N compared to controls and IgAV-noN ($p < 0.001$). Concentrations also

Table 1 (continued)

Author	Year	Study design	Cohort demographic	Definition of nephritis	Type of sampling	Laboratory technique	Biomarker	Results
			(M = 71, F = 64) and 84 healthy controls (M = 48, F = 36).	grouped into mild/-moderate/severely high urinary protein concentration.				increased in parallel with the degree of urinary protein concentration (all $p < 0.01$).
Wang et al. [22]	2017	Prospective longitudinal	35 children (M = 18, F = 17) with IgAV-N, 41 paediatric patients (M = 18, F = 23) with a diagnosis of IgAV-noN and 32 healthy controls (M = 17, F = 15).	Haematuria and/or high urinary protein concentration within 6 months after the onset of rash.	Midstream first morning urine sample before and after treatment	ELISA	Macrophage migration inhibitory factor (MIF)	Urinary MIF was greatest in group I and higher than group II or controls (both $p < 0.05$).
Ye et al. [23]	2015	Prospective cross sectional	694 children (M = 332, F = 362) with biopsy-proven IgAV-N, compared to 400 healthy controls (M = 188, F = 212).	Nephritis was graded according to the KDIGO criteria. Biopsy was classified according the ISKDC criteria.	N/A[b]	Roche Modular P800 biochemical analyser	24-h urinary protein (24h-UPRO) Urinary protein:Cr ratio (U-PCR)	There was an increase in 24-UPRO and U-PCR when comparing those with grades I or IIa to grades IIb, IIIa or IIIb ($p < 0.01$). 24-UPRO was increased in IgAV-N compared to controls ($p < 0.01$).
Zhang et al. [24]	2015	Prospective longitudinal	27 children with IgAV-noN (M = 19, F = 8) were compared to 32 paediatric patients with IgAV-N (M = 18, F = 14) and 16 healthy volunteers (M = 9, F = 7).	Those who underwent a kidney biopsy were graded according to ISKDC criteria.[c]	Spot morning urine samples	ELISA	Kidney injury molecule-1 (KIM-1) N-Acetyl-beta-glucosaminidase (NAG) Beta-2 microglobulin (β2-MG)	Urinary KIM-1 concentrations were increased in IgAV-N compared to IgAV and controls ($p < 0.05$). Patients with IgAV had an increased concentration of urinary KIM-1 compared to controls ($p < 0.001$). NAG was highest in IgAV-N ($p < 0.05$).

Abbreviations: *Cr*, creatinine; *eGFR*, estimated glomerular filtration rate; *ELISA*, enzyme-linked immunosorbent assay; *Ig*, immunoglobulin; *IgAV*, immunoglobulin A vasculitis; *IgAV-N*, immunoglobulin A vasculitis nephritis; *IgAV-noN*, immunoglobulin A vasculitis without nephritis; *IL*, interleukin; *ISKDC*, International Study of Kidney Disease in Children; *KDIGO*, Kidney Disease Improving Global Outcomes; *PCR*, protein:creatinine ratio; *UC*, urinary creatinine; *UP*, urinary protein

[a] As this study was not published in English, data was only extracted from the abstract and this information was not available

[b] Method of urine sampling was not specified

[c] Nephritis was not defined in this study

descriptive statistics will be presented as percentage male and a median age will be calculated using the available age data. Laboratory data will be presented as either a mean with standard deviation or as a median with range depending on the original publication. Area under the curve (AUC) will be presented to represent the strength of the biomarker and described as a value from 0–1.0 with a 95% confidence interval. In terms of biomarker strength, an AUC of ≤ 0.5 suggests no discrimination, 0.7–0.8 is considered acceptable, 0.8–0.9 is considered excellent, and ≥ 0.9 is considered outstanding [26]. *p*-values < 0.05 and a confidence interval which does not overlap 0 will be considered significant. As it was expected that the studies revealed would be heterogeneous, a meta-analysis was not conducted.

Ethical approval

Ethical approval was not necessary for the performance of this review, as per the National Health Service Research Authority, as it involved secondary review of existing literature.

Results

Data extraction

The search took place in September 2020 and yielded 121 papers. A total of 65 duplicates were removed leaving 56 titles eligible for abstract screening. Of these, 26 papers were eligible for full text review. After full text review, 11 were included in the systematic review. A second, independent reviewer (AT) repeated the search, at a time point 1 month later, to identify papers and determined whether the studies met the inclusion criteria; 128 papers were retrieved and after deduplication, two additional papers were identified that met the inclusion criteria, producing a total of 13 papers (Fig. 1). No further eligible papers were discovered in searching the reference lists.

Participants

A total cohort of 2446 children were included in this systematic review from 13 studies. The median age of the entire cohort was 7.9 years and 51% were male. Data on sex was not available in one study [12]. Median or mean age was not available in two papers [12, 15] and age ranges could not be calculated due to the heterogeneity of the papers in presenting demographic data.

The participants comprised 1236 children with IgAV-N (48% male, median age 8.0 years), 761 healthy paediatric controls (52% male, median age 7.9 years) and 449 children with IgAV-noN (52% male, median age 7.0 years). The publication dates spanned from 2011–2020 [13, 14, 17, 27] and included both longitudinal [13, 17, 18, 24, 28, 29] and cross-sectional studies [12, 14, 15, 19, 22, 23, 27]. The majority of the papers were published from China [12, 14, 17, 18, 22–24, 27, 28, 30], and three studies were from Poland [13], France [19] and Mexico [15].

Quality appraisal

The quality appraisal produced a good median AXIS score of 16/20 (range 14–17). One study was excluded from the quality assessment as it was not available in full text in English and there was insufficient detail in the abstract [17]. Those studies with lower AXIS scores were mostly due to small sample size, single site recruitment, and no mention of study limitations.

Identified biomarkers

A total of 23 urine biomarkers were discovered that had been reported to be associated with IgAV-N; 20 were pre-clinical and 3 considered clinical biomarkers (Table 2). Increased urinary protein concentration was the only clinical urine biomarker identified and had been measured using 24-h urinary protein (24h-UPRO) values, urinary protein:creatinine ratio (U-PCR) and urinary albumin concentration (Malb). There were 5 pre-clinical urine biomarkers that had been reported more than once and thus described in more detail, these were as follows: beta-2 microglobulin (β2-MG), kidney injury molecule-1 (KIM-1), monocyte chemoattractant protein-1 (MCP-1), N-acetyl-β-glucosaminidase (NAG) and urinary angiotensinogen (UAGT).

Urinary protein concentration

(i) Presence of nephritis: As expected, the 24h-UPRO was significantly increased in children with biopsy-proven IgAV-N (n = 694) compared to healthy controls (n = 400; $p < 0.01$). In a second paper, the urine Malb concentration was significantly increased in the IgAV-N group ($n = 37$) compared to both healthy controls and the IgAV-noN cohorts ($p < 0.05$) and the control group ($n = 37$) was not significantly different to the IgAV-noN patients (n = 34, $p > 0.05$) [16].

(ii) Severity of nephritis: Importantly, differences could be seen within the IgAV-N cohort when comparing histological grades I and IIa versus IIb, IIIa and IIIb (all $p <$ 0.01). The AUC value was 0.77 for 24h-UPRO as a biomarker in distinguishing histology grades IIb, IIIa and IIIb. UPCR was also evaluated when assessing the severity of nephritis producing an AUC value of 0.73 [23]. Malb positively correlated with the grading of IgAV-N (n = 45, $p < 0.05$), with excellent AUC values for histological comparison (grade I vs. II AUC 0.95, 95% CI 0.87–1.00; grade II vs. III AUC 0.81, 95% CI 0.66–0.95; grade I vs. III AUC 0.98, 95% CI 0.94–1.00) [12].

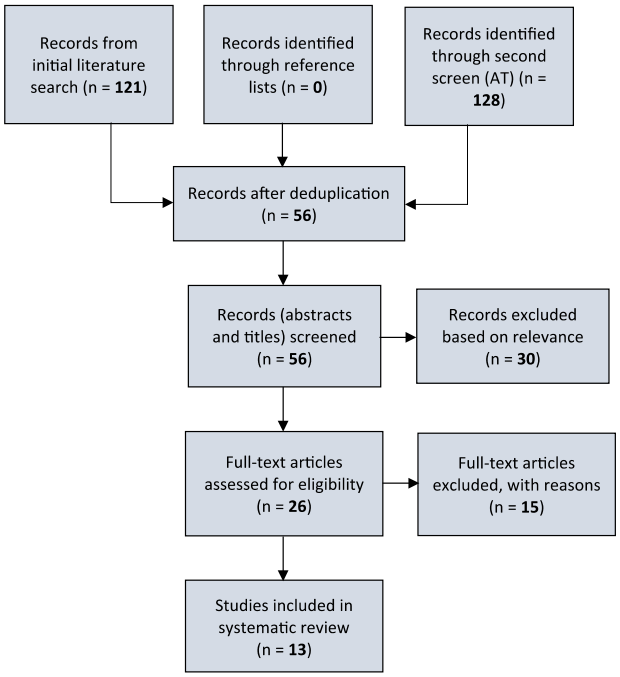

Fig. 1 A flow diagram to represent the search and screen process. The systematic literature search was performed on 4 databases and returned 121 papers. Fifty-six papers were identified after deduplication. After screening by initial and a second independent person, a total of 13 studies were included in the systematic review

Urinary β2-MG

(i) Presence of nephritis: One paper found that urine β2-MG was significantly increased in IgAV-N patients (n = 37) compared to both healthy controls (n = 37) and IgAV-noN (n = 34, $p < 0.05$) [16]. Qin et al. reported statistically significantly increased urinary concentration of β2-MG in children with IgAV-N (n = 66) compared to children with IgAV-noN (n = 68, $p < 0.05$) [20].

(ii) Severity of nephritis: Another paper (IgAV-N, n = 45) compared urinary β2-MG with the histological grades, grouped according to the ISKDC classification [10]. They found that urinary β2-MG was statistically significantly increased in all groups ($p < 0.05$) with no statistical difference between the histological classifications [12]. Zhang et al. explored urinary β2-MG in predicting irreversible kidney damage (defined as histological changes according to the ISKDC criteria) and reported a poor AUC at 0.49 (95% CI = 0.35–0.63, $p = 0.89$) [24].

Urinary KIM-1

(i) Presence of nephritis: This was reported as a potential biomarker in two studies. Dyga et al. found that KIM-1 was statistically significantly increased acutely in all IgAV patients ($n = 29$) when compared to the controls ($p < 0.005$) but there was no significant difference between IgAV-noN (n = 18) and IgAV-N (n = 11). Urinary KIM-1 concentrations decreased over time in IgAV-N and IgAV-noN [13]. Zhang et al. found the contrary, with mean urinary KIM-1 concentrations significantly increased in IgAV-N (n = 32) compared to IgAV-noN ($n = 27$, $p < 0.05$) and healthy controls (n = 16, $p < 0.05$). The AUC for KIM-1 in predicting nephritis was outstanding at 0.93 (95% CI = 0.88–0.99, $p <$ 0.05) [24].

(ii) Severity of nephritis: A positive correlation between urinary KIM-1 levels and histological grade or total urine protein was found ($r = 0.671$, $p < 0.01$) [24]. Another paper found no statistical difference in distinguishing severity [13].

Urinary MCP-1

(i) Presence of nephritis: This was found to correlate with IgAV-N in two studies, reporting 447 children. Fuentes et al. reported a statistically significantly increased urinary MCP-1/Cr concentration in the IgAV-N cohort (n = 57) compared to healthy controls ($n = 25$) or IgAV-noN (n = 27, $p < 0.01$) [15]. Wang et al. also found urinary

Table 2 Frequency of biomarker identification in this systematic review

Biomarker identified	Studies
Beta-2 microglobulin (β2-MG)	An and Xia [12] Ge et al. [28] Qin et al. [27] Zhang et al. [24]
24-h urinary protein (24h-UPRO)	Ye et al. [23]
Fibroblast specific protein-1 (FSP-1)	Ma et al. [17]
Immunoglobulin λ/immunoglobulin K ratio (IgλIgK ratio)	Pillebout et al. [19]
Immunoglobulin A/Cr ratio (IgA/Cr)[a]	Pillebout et al. [19]
Immunoglobulin G/Cr ratio (IgG/Cr)[a]	Pillebout et al. [19]
Immunoglobulin M/Cr ratio (IgM/Cr)[a]	Pillebout et al. [19]
Interleukin-6/Cr ratio (IL-6/Cr)[a]	Pillebout et al. [19]
Interleukin-8/Cr ratio (IL-8/Cr)[a]	Pillebout et al. [19]
Interleukin-10/Cr ratio (IL10/Cr)[a]	Pillebout et al. [19]
Integrin beta-1 (ITGB1)	Fang et al. [14]
Kidney injury molecule-1 (KIM-1)	Dyga et al. [13] Zhang et al. [24]
Liver-fatty acid binding protein (L-FABP)	Dyga et al. [13]
Urinary albumin concentration (Malb)	An and Xia [12] Ge et al. [28]
Monocyte chemoattractant protein-1 (MCP-1)	Fuentes et al. [15] Wang et al. [22]
Macrophage migration inhibitory factor (MIF)	Wang et al. [29]
Matrix metalloproteinase-9 (MMP-9)	Qin et al. [27]
N-Acetyl-beta-glucosaminidase (NAG)	An and Xia [12] Zhang et al. [24]
Neutrophil gelatinase-associated lipocalin (NGAL)	Dyga et al. [13]
Transferrin (TfR)	An and Xia [12]
Tissue inhibitor matrix metalloproteinase-1 (TIMP-1)	Qin et al. [27]
Urinary angiotensinogen (UAGT)	Ma et al. [17]
Urinary protein:Cr ratio (U-PCR)[a]	Ye et al. [23]

[a] Cr refers to creatinine

MCP-1 to be significantly increased in IgAV-N ($n = 126$) compared to healthy controls ($n = 84$, $p < 0.01$) and IgAV-noN ($n = 135$, $p < 0.01$). Urine MCP-1 concentrations increased in parallel with the degree of urinary protein concentration [21].

(ii) Severity of nephritis: One paper found that the AUC for MCP-1 predicting nephritis was excellent (AUC 0.83 95% CI = 0.73–0.92, $p < 0.01$) [15].

Urinary NAG

(i) Presence of nephritis: Zhang et al. also found increased urinary NAG concentration in IgAV-N ($n = 32$) compared to IgAV-noN ($n = 27$, $p < 0.05$). There was no difference between IgAV-noN ($n = 27$) and healthy controls ($n = 16$). The AUC for urinary NAG in distinguishing patients with nephritis was excellent (AUC 0.82 95% CI 0.72–0.92, $p < 0.01$) [24].

(ii) Severity of nephritis: An and Xia evaluated urinary NAG in biopsy-proven IgAV-N ($n = 45$). The concentrations correlated with increasing histological grade ($p < 0.05$) and the AUC in predicting the histological grades were excellent for grade I vs. II (AUC 0.84 95% CI 0.67–1.00), outstanding for grade I vs. III (AUC 0.96 95% CI 0.89–1.00); and acceptable for grade II vs. III (AUC 0.76 95% CI 0.59–0.93) [12].

Urinary angiotensinogen (UAGT)

(i) Presence of nephritis: Ma et al. compared IgAV-N ($n = 14$), IgAV-noN ($n = 28$) and healthy controls ($n = 23$). UAGT/Cr was significantly increased in IgAV-N compared to healthy controls and IgAV-noN ($p < 0.05$). This paper was unavailable in full text in English so limited data was extracted from the abstract only [17]. Mao et al. further subdivided patients with IgAV-N and described acute increase in UAGT in IgAV-N patients with a high urinary protein concentration ($n = 13$) compared to both IgAV-noN ($n = 51$) and IgAV-N with only haematuria ($n = 43$, $p < 0.01$). This finding remained even during the convalescent phase where UAGT concentrations remained increased in the IgAV-N with a high urinary protein concentration compared to the IgAV-noN ($p < 0.01$) and the IgAV-N with haematuria ($p < 0.01$). The difference in concentration during the convalescent phase between the IgAV-noN and IgAV-N with haematuria was not significant [18].

(ii) Severity of nephritis: No studies assessed UAGT to determine the severity of nephritis.

Discussion

This systematic review aimed to identify current clinical and potential pre-clinical urine biomarkers associated with the presence of nephritis and its severity in children with IgAV-N. Using a predetermined systematic evaluation, we have reported a cohort of 2446 children, including 1685 children with IgAV, using data from 13 papers. These data identified 23 potential biomarkers described in the literature including the clinical biomarker of urinary protein concentration and 5 pre-clinical urine biomarkers that had been evaluated by more than one study. Of these pre-clinical biomarkers, 4 demonstrated promising association with IgAV nephritis: KIM-1,

MCP-1, NAG and UAGT [13, 15, 17, 18, 22, 24]. One urine biomarker, β2-MG, although frequently studied, did not perform well [12, 16, 24]. A further 18 markers were less frequently reported but were summarised as they may have potential future utility in this disease and provide important insight into the underlying pathophysiology.

The clinical biomarker that performed best at assessing the severity of nephritis was urinary albumin concentration with excellent AUC values (AUC 0.81–0.98) in determining the grade of histological inflammation in IgAV-N. The preclinical biomarkers, KIM-1, MCP-1, NAG and UAGT, demonstrate promise for their association with either the presence or severity of nephritis, and their relative advantages and disadvantages are summarised in Table 3.

In addition to highlighting promising biomarkers, this study provides insight into key biological pathways in IgAV-N. The fact that many of the most promising biomarkers arise as a result of tubulointerstitial inflammation is an extremely interesting finding as IgAV-N is traditionally considered solely a glomerulonephritis. Examples of these markers are KIM-1 and NAG. KIM-1 is a type 1 transmembrane protein that is absent in the normal kidney, upregulated in tubular injury and not expressed in other organs [33]. It is a recognised biomarker in acute tubular necrosis and allograft nephropathy where it has been found to correlate with the degree of tubulointerstitial insult [34–36]; however, it has not yet been reported in the histology for IgAV-N. This review included one small study that found no clear relationship between KIM-1 concentration and IgAV-N but it did demonstrate a reduction over time suggesting some relationship with disease activity [13]. A larger study by Zhang et al. reported an outstanding AUC (0.93) for KIM-1 in its ability to identify

Table 3 A table comparing the clinical and pre-clinical biomarkers, their AUC values and their advantages and disadvantages

Biomarker		AUC values	Region of kidney predominantly released from	Advantages	Disadvantages
Urinary protein concentration	Urinary albumin concentration	0.81–0.98	Glomerulus	• Established marker of disease • Available in clinical laboratories	• Only present when damage has already occurred as it is a sign of kidney damage • Albuminuria superior to proteinuria • 24-UPRO rarely performed in practice
	24-h urinary protein (24h-UPRO) or protein:creatinine ratio (PCR)	0.73–0.77	Glomerulus	• Associated with prediction of severity of nephritis	
Kidney injury molecule-1 (KIM-1)		0.93	Tubulointerstitial	• Not expressed in other organs so very specific • Outstanding AUC • Has been suggested to correlate with IgAV-N and IgA nephropathy in the adult population where correlation with the degree of tubulointerstitial injury was also reported [31, 32]	• May only be released due to downstream result of glomerular damage • One paper found no clear relationship • Not yet an established marker of disease • Not reported to correlate with histology
Monocyte chemoattractant protein-1 (MCP-1)		0.83	Glomerular	• Reported to provide early identification of nephritis and predict histology in two papers • Associated with histology • Previously found to be associated with IgA nephropathy and lupus nephritis in adult populations	• Not yet an established marker of disease
N-Acetyl-beta-glucosaminidase (NAG)		0.82	Tubular	• Early identification of nephritis and predictive potential, able to correlate with histology	• Few previous studies on IgA-mediated diseases • Not yet an established marker of disease • May only be released due to downstream result of glomerular damage
Urinary angiotensinogen (UAGT)		n/a	Glomerular and/or tubular	• May imply novel pathophysiology not previously studied	• No AUC value to compare • Not yet an established marker of disease • If tubular involvement, may only be released due to downstream result of glomerular damage

IgAV-N [37, 38]. The lysosomal enzyme NAG is found in many body tissues, but it is found in particularly high concentrations in the proximal kidney tubular cells. NAG may be released into the urine via exocytosis or, more commonly, during kidney injury causing proximal tubule leakage [39]. Urinary NAG has been described in patients with acute kidney injury and more recently in diabetic nephropathy; however, there are few studies in IgA-mediated kidney diseases [40–42]. Our review found urinary NAG as a promising biomarker, able to distinguish patients with IgAV-N from those without nephritis [37] and accurately correlate with the degree of histopathology in IgAV-N [12]. This suggests that tubular inflammation may play a larger role than previously thought and warrants further evaluation. Tubular markers may be evident due to tubular damage leading to urinary release of these proteins as a downstream result of glomerular damage or from direct tubular involvement. Tubulointerstitial components have recently been added to proposed histological scoring classification systems for IgAV-N due to their better correlation with clinical outcomes. This supports the finding that the tubulointerstitial region may be of importance in this disease [43].

Nephritis is the main long-term complication of IgAV and there is currently no way to predict and identify which children may get irreversible kidney damage from the outset, thus all children are committed to a period of at least 6 months of monitoring. A better understanding of the underlying biology represented by urine biomarkers may allow identification of children who are at low or high risk of disease progression allowing monitoring stratification from the outset. Further studies are required to demonstrate whether pre-clinical markers are superior to current clinical biomarkers in terms of their ability to earlier detect nephritis or predict severity.

Limitations of this study include some studies being small and the heterogeneous nature of the papers regarding descriptive statistics, definition of nephritis, and type of sampling, methodologies, outcomes and data presentation made comparisons challenging. This review has identified the need for standardisation of biomarker evaluation in this disease to allow systematic comparison in the future. Some papers had missing data and one was only available in abstract form in English. The majority of these studies were cross sectional in design, so future longitudinal studies are needed to evaluate how the biomarkers change with the course of disease. Finally, most of the papers included in our review were from China and the relevance of ethnic variation of the expression of urinary biomarkers is currently unknown.

Conclusion

Overall, this study suggests that there are promising urine biomarkers for IgAV-N and some of these also originate from the tubulointerstitial region suggesting a pathophysiological role. In order to assess their true potential as adjuncts to clinical practice, long-term evaluation of these urine biomarkers is needed.

Author contribution All authors declare that this is an original manuscript and that they meet the criteria for authorship.

Declarations

Conflict of interest The authors declare no competing interests.

References

1. Gardner-Medwin JM, Dolezalova P, Cummins C, Southwood TR (2002) Incidence of Henoch-Schönlein purpura, Kawasaki disease, and rare vasculitides in children of different ethnic origins. Lancet 360:1197–1202
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 CGM, Lamprecht P, Langford CA, Luqmani RA, Mahr AD, Matteson EL, Merkel PA, Ozen S, Pusey CD, Rasmussen N, Rees AJ, Scott DGI, Specks U, Stone JH, Takahashi K, Watts RA (2013) 2012 revised international Chapel Hill consensus conference nomenclature of vasculitides. Arthritis Rheum 65:1–11
3. Heineke MH, Ballering AV, Jamin A, Ben Mkaddem S, Monteiro RC, Van Egmond M (2017) New insights in the pathogenesis of immunoglobulin A vasculitis (Henoch-Schönlein purpura). Autoimmun Rev 16:1246–1253
4. Blanco R, Martínez-Taboada VM, Rodríguez-Valverde V, García-Fuentes M, González-Gay MA (1997) Henoch-Schönlein purpura in adulthood and childhood: two different expressions of the same syndrome. Arthritis Rheum 40:859–864
5. Nong BR, Huang YF, Chuang CM, Liu CC, Hsieh KS (2007) Fifteen-year experience of children with Henoch-Schönlein purpura in southern Taiwan, 1991–2005. J Microbiol Immunol Infect 40:371–376
6. Jauhola O, Ronkainen J, Koskimies O, Ala-Houhala M, Arikoski P, Hölttä T, Jahnukainen T, Rajantie J, Ormälä T, Nuutinen M (2010) Clinical course of extrarenal symptoms in Henoch-Schonlein purpura: a 6-month prospective study. Arch Dis Child 95:871–876
7. Oni L, Sampath S (2019) Childhood IgA vasculitis (Henoch Schonlein purpura)-advances and knowledge gaps. Front Pediatr 7:257

8. Narchi H (2005) 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 90:916–920
9. 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 (2010) 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 69:798–806
10. Huang X, Ma L, Ren P, Wang H, Chen L, Han H, Chen J, Han F (2019) Updated Oxford classification and the international study of kidney disease in children classification: application in predicting outcome of Henoch-Schönlein purpura nephritis. Diagn Pathol 14: 40
11. Downes MJ, Brennan ML, Williams HC, Dean RS (2016) Development of a critical appraisal tool to assess the quality of cross-sectional studies (AXIS). BMJ Open 6:e011458
12. An JK, Xia D (2018) Diagnostic performance of urinary proteins as biomarkers in evaluating Henoch Schonlein purpura nephritis. Clin Exp Med 11:12354–12360
13. Dyga K, Machura E, Świętochowska E, Szczepańska M (2020) Analysis of the association between kidney injury biomarkers concentration and nephritis in immunoglobulin A vasculitis: a pediatric cohort study. Int J Rheum Dis 23:1184–1193
14. Fang X, Wu HY, Lu M, Cao Y, Wang R, Wang MQ, Gao CL, Xia ZK (2020) Urinary proteomics of Henoch-Schonlein purpura nephritis in children using liquid chromatography-tandem mass spectrometry. Clin Proteomics 17:10
15. Fuentes Y, Hernández AM, García-Roca P, Valverde S, Velásquez-Jones LF, Sosa G, Duarte-Durán UO, Ortíz L, Maldonado R, Faugier E, Ramón-García G, Medeiros M (2014) Urinary MCP-1/creatinine in Henoch-Schönlein purpura and its relationship with nephritis. Pediatr Nephrol 29:1047–1052
16. Ge W, Wang H-L, Sun R-P (2014) Pentraxin 3 as a novel early biomarker for the prediction of Henoch-Schonlein purpura nephritis in children. Eur J Pediatr 173:213–218
17. Ma YF, Li YF, Guo GM, Zhu YJ, Gong YL, Dong Y (2020) Changes of new urinary biomarkers in children with Henoch-Schonlein purpura nephritis. J Shanghai Jiaotong Univ Med Sci 40:842–846
18. Mao YN, Liu W, Li YG, Jia GC, Zhang Z, Guan YJ, Zhou XF, Liu YF (2012) Urinary angiotensinogen levels in relation to renal involvement of Henoch-Schonlein purpura in children. J Nephrol 17: 53–57
19. Pillebout E, Jamin A, Ayari H, Housset P, Pierre M, Sauvaget V, Viglietti D, Deschenes G, Monteiro RC, Berthelot L (2017) Biomarkers of IgA vasculitis nephritis in children. PLoS One 12: e0188718
20. Qin Y-H, Zhou T-B, Lei F-Y, Huang W-F, Zhao Y-J, Lin F-Q, Su L-N (2011) Cut-off values for serum matrix metalloproteinase-9: is there a threshold to predict renal involvement for Henoch-Schonlein purpura in children? Nephrology (Carlton) 16:93–99
21. Wang J, Ying Q, Zhong S, Chen Y, Di Y, Dai X, Zheng J, Shen M (2018) Elevated urinary monocyte chemoattractant protein-1 levels in children with Henoch-Schonlein purpura nephritis. Pediatr Neonatol 59:238–243
22. Wang J, Ying Q, Zhong S, Chen Y, Di Y, Dai X, Zheng J, Shen M (2017) Elevated urinary monocyte chemoattractant protein-1 levels in children with Henoch-Schonlein purpura nephritis. Pediatr Neonatol 59:238–243
23. Ye Q, Shang SQ, Liu AM, Zhang T, Shen HQ, Chen XJ, Mao JH (2015) 24h urinary protein levels and urine protein/creatinine ratios could probably forecast the pathological classification of HSPN. PLoS One 10:e0127767
24. Zhang J, Zeng H, Wang N, Tian X, Dou W, Shi P (2015) Beneficial effects of creatine phosphate sodium for the treatment of Henoch-Schönlein purpura in patients with early renal damage detected using urinary kidney injury molecule-1 levels. Eur J Pediatr 175: 49–55
25. Califf RM (2018) Biomarker definitions and their applications. Exp Biol Med (Maywood) 243:213–221
26. Mandrekar JN (2010) Receiver operating characteristic curve in diagnostic test assessment. J Thorac Oncol 5:1315–1316
27. Qin YH, Zhou TB, Lei FY, Huang WF, Zhao YJ, Lin FQ, Su LN (2011) Cut-off values for serum matrix metalloproteinase-9: is there a threshold to predict renal involvement for Henoch-Schonlein purpura in children? J Nephrol 16:93–99
28. Ge W, Wang HL, Sun RP (2014) Pentraxin 3 as a novel early biomarker for the prediction of Henoch-Schönlein purpura nephritis in children. Eur J Pediatr 173:213–218
29. Wang JP, Li YY, Chen YL, Dai XH, Di YZ, Shen MJ, Ying QQ, Fu SW, Li YJ (2017) Urinary macrophage migration inhibitory factor as a noninvasive biomarker in pediatric Henoch-Schonlein purpura nephritis. J Clin Rheumatol 23:258–261
30. Wang J, Li Y, Chen Y, Dai X, Di Y, Shen M, Ying Q, Fu S, Li Y (2017) Urinary macrophage migration inhibitory factor as a noninvasive biomarker in pediatric Henoch-Schonlein purpura nephritis. J Clin Rheumatol 23:258–261
31. Zhang Y, Li A, Wen J, Zhen J, Hao Q, Zhang Y, Hu Z, Xiao X (2017) Kidney injury molecule-1 level is associated with the severity of renal interstitial injury and prognosis in adult Henoch-Schönlein purpura nephritis. Arch Med Res 48:449–458
32. Xu P-C, Zhang J-J, Chen M, Lv J-C, Liu G, Zou W-Z, Zhang H, Zhao M-H (2011) Urinary kidney injury molecule-1 in patients with IgA nephropathy is closely associated with disease severity. Nephrol Dial Transplant 26:3229–3236
33. Song J, Yu J, Prayogo GW, Cao W, Wu Y, Jia Z, Zhang A (2019) Understanding kidney injury molecule 1: a novel immune factor in kidney pathophysiology. Am J Transl Res 11:1219–1229
34. Han WK, Bailly V, Abichandani R, Thadhani R, Bonventre JV (2002) Kidney injury molecule-1 (KIM-1): a novel biomarker for human renal proximal tubule injury. Kidney Int 62:237–244
35. Edelstein CL (2017) Chapter six - biomarkers in acute kidney injury. In: Edelstein CL (ed) Biomarkers of kidney disease (Second Edition). Academic Press, pp 241-315
36. Waanders F, van Timmeren MM, Stegeman CA, Bakker SJL, van Goor H (2010) Kidney injury molecule-1 in renal disease. J Pathol 220:7–16
37. Zhang J, Zeng H, Wang N, Tian X, Dou W, Shi P (2016) Beneficial effects of creatine phosphate sodium for the treatment of Henoch-Schönlein purpura in patients with early renal damage detected using urinary kidney injury molecule-1 levels. Eur J Pediatr 175: 49–55
38. Liu F, Wang C, Wang R, Wang W, Li M (2018) Henoch-Schonlein purpura nephritis with renal interstitial lesions. Open Med (Wars) 13:597–604
39. Wen X, Kellum JA (2012) N-Acetyl-beta-D-glucosaminidase (NAG). In: Vincent J-L, Hall JB (eds) Intensive care med. Springer, Berlin Heidelberg, Berlin, Heidelberg, pp 1509–1510
40. Vaidya VS, Ferguson MA, Bonventre JV (2008) Biomarkers of acute kidney injury. Annu Rev Pharmacol Toxicol 48:463–493
41. Sheira G, Noreldin N, Tamer A, Saad M (2015) Urinary biomarker N-acetyl-β-D-glucosaminidase can predict severity of renal damage in diabetic nephropathy. Diabetes Metab Syndr 14:4

42. Bazzi C, Petrini C, Rizza V, Arrigo G, Napodano P, Paparella M, D'Amico G (2002) Urinary N-acetyl-beta-glucosaminidase excretion is a marker of tubular cell dysfunction and a predictor of outcome in primary glomerulonephritis. Nephrol Dial Transplant 17: 1890–1896
43. Koskela M, Ylinen E, Ukonmaanaho EM, Autio-Harmainen H, Heikkilä P, Lohi J, Jauhola O, Ronkainen J, Jahnukainen T, Nuutinen M (2017) The ISKDC classification and a new semiquantitative classification for predicting outcomes of Henoch-Schönlein purpura nephritis. Pediatr Nephrol 32:1201–1209

Appendix 2 | The PVAS score used to score the disease activity in IgAV patients to test concurrent validity.

PAEDIATRIC VASCULITIS ACTIVITY SCORE					
○ Tick "**Active**" box **only** if abnormality due to active vasculitis is newly present or worse over the last 4 weeks or persists for less than 3 months. After that, if ALL items are persistent and represent smouldering/low grade/grumbling disease, and there are no new/worse features, please tick the box at the bottom right corner. At the very first assessment all active items are considered as active/worse. If there are no abnormalities in a system, please tick the "**None**" box. For items present longer than 3 months refer to the Vasculitis Damage Index to score damage.					
	None	**Active**		**None**	**Active**
1. General	○		**6. Cardiovascular**	○	
Myalgia		○	Loss of pulses		○
Arthralgia or arthritis		○	Bruits over accessible arteries		○
Fever ≥38.0°C		○	Blood pressure discrepancy		○
Weight loss ≥5% body weight		○	Claudication of extremities		○
2. Cutaneous	○		Ischaemic cardiac pain		○
Polymorphous exanthem		○	Cardiomyopathy		○
Livdeo		○	Congestive cardiac failure		○
Panniculitis		○	Valvular heart disease		○
Purpura		○	Pericarditis		○
Skin nodules		○	**7. Abdominal**	○	
Infarct (nail edge lesion, splinter haemorrhage)		○	Abdominal pain		○
Ulcer (full-thickness necrosis)		○	Peritonitis		○
Gangrene (extensive necrosis)		○	Blood in stools or bloody diarrhoea		○
Other skin vasculitis (specify below)		○	Bowel ischaemic		○
3. Mucous membranes/eyes	○		**8. Renal**	○	
Mouth ulcers/granulomata		○	Hypertension >95th centile (for height)		○
Genital ulcers		○	Proteinuria >0.3g/24h, >20mmol/mg creatinine		○
Adnexal inflammation		○	Haematuria ≥2+ or 5 rbc/hpf or red cell casts		○
Significant proptosis		○	GFR 50-80ml/min/1.73m^2		○
Red eye (epi)scleritis		○	GFR 15-49ml/min/1.73m^2		○
Red eye conjunctivitis/blepharitis/keratitis		○	GFR <15ml/min/1.73m^2		○
Uveitis		○	Rise in creatinine >10% or creatinine clearance (GFR) fall >25%		○
Blurred vision		○	**9. Nervous system**	○	
Sudden visual loss		○	Headache		○
Retinal vasculitis/retinal vessel thrombosis/retinal exudates/haemorrhages		○	Meningitis/encephalitis		○
4. ENT	○		Organic confusion/cognitive dysfunction		○
Nasal discharge/crusts/ulcers/granuloma		○	Seizures (not hypertensive)		○
Paranasal sinus involvement		○	Stroke		○

Subglottic stenosis/hoarseness/stridor	○	Cord lesion	○
Conductive hearing loss	○	Cranial nerve palsy	○
Sensorineural hearing loss	○	Sensory peripheral neuropathy	○
5. Chest	○	Motor mononeuritis multiplex	○
Wheeze or expiratory dyspnoea	○	**10. OTHER**	○
Endobronchial/endotracheal involvement	○		○
Nodules or cavities	○	**NO NEW/WORSE DISEASE:**	
Pleural effusion/pleurisy	○	Tick here if there is no new/worse abnormality present in ANY of the systems above and active items represent low grade grumbling disease/	
Infiltrate	○		
Massive haemoptysis/alveolar haemorrhage	○		
Respiratory failure	○		

Glossary and scoring for PVAS. GENERAL RULE: disease features are scored only when they are due to active vasculitis, after excluding other causes (e.g. infection, hypertension, etc.). If the feature is due to active disease, it is scored in the boxes. It is essential to apply these principles to each item below. Scores have been weighted according to the severity which each symptom or sign is thought to represent. Tick "Persistent Disease" box if all the abnormalities are due to active (but not new or worse) vasculitis. If any of the abnormalities are due to new/worse disease, DO NOT tick the "Persistent Disease" box. For some features, further information (from specialist opinion or further tests) is required if abnormality is newly present or worse. Remember that in most instances, you will be able to complete the whole record when you see the patient. However, you may need further information before entering some items. Please leave these items blank, until the information is available, and then fill them in. For example, if the patient has new onset of stridor, you would usually ask an ENT colleague to investigate this further to determine whether or not it is due to active Wegener's granulomatosis.		**PVAS persistent**	**PVAS new/worse**
1. General	**Maximum scores**	**2**	**3**
Myalgia	Diffuse, spontaneous, hard to localize muscle pain or tenderness on muscle palpatıon. Exclude fibromyalgia.	1	1
Arthralgia or arthritis	Joint pain in any number of joints or presence of objective signs of active synovitis: intraarticular swelling due to synovial proliferation and/or joint effusion with limited range of movement and/or pain on movement or joint tenderness. Any number of joints.	1	1
Fever ≥38.0°C	Documented temperature elevation >38oC. The value refers to axillary/oral temperature (rectal temperature 0.5°C higher). Exclude infections by appropriate cultures, serology and PCR methods.	2	2
Weight loss ≥5% body weight	At least 5% loss of body weight (not fluid) having occurred since last assessment or in the 4 weeks not as a consequence of dieting.	2	2
2. Cutaneous	**Maximum scores**	**3**	**6**
Polymorphous exanthem	Non-haemorrhagic, non-necrotising skin eruption of any type or combined types. Exclude allergy/drug reaction/infection.	1	1
Livdeo	Purplish reticular pattern usually irregularly distributed around subcutaneous fat lobules, often more prominent with cooling, common over foot margins. Exclude antiphospholipid syndrome.	1	1
Panniculitis	Single or multiple tender deep subcutaneous nodules caused by inflammation of deep subcutaneous tissue with typical histopathology findings if biopsy performed.	1	1

Purpura	Petechiae (small red spots), palpable purpura, or ecchymoses (large plaques) in skin or oozing (in the absence of trauma) in the mucous membranes.	1	2
Skin nodules	Subcutaneous nodules, often along arteries, tender on palpation.	1	1
Infarct (nail edge lesion, splinter haemorrhage)	Nail edge lesion, splinter haemorrhage or flea bite lesion of small vessel vasculitis.	1	1
Ulcer (full-thickness necrosis)	Area of full-thickness skin/subcutaneous tissue ulceration/necrosis.	1	4
Gangrene (extensive necrosis)	Extensive skin/subcutaneous tissue/underlying structure necrosis, digital phalanx or other peripheral (nose, ear tips) necrosis/gangrene.	2	6
Other skin vasculitis (specify below)	Vasculitis different from previous e.g. subcutaneous swelling/oedema due to capillary leak in small vessel involvement, Raynaud´s phenomenon etc.	1	1
3. Mucous membranes/eyes	**Maximum scores**	**3**	**6**
Mouth ulcers/granulomata	Aphthous stomatitis, ischaemic ulcers and/or granulomatous inflammation in oral cavity. Exclude other causes (SLE, infection).	1	2
Genital ulcers	Ulcers localised in the genitalia or perineum, excluding infections.	1	2
Adnexal inflammation	Salivary (diffuse, tender swelling unrelated to meals) or lacrimal gland inflammation. Exclude other causes (infection). Specialist opinion preferably required.	2	4
Significant proptosis	Protrusion of the eyeball due to significant amounts of inflammatory in the orbit; if unilateral, there should be a difference of 2 mm between one eye and the other. This may be associated with diplopia due to infiltration of extra-ocular muscles. Developing myopia (measured on best visual acuity, see later) can also be a manifestation of proptosis.	2	4
Red eye (epi)scleritis	Inflammation of the sclerae (specialist opinion usually required). Can be heralded by photophobia.	1	2
Red eye conjunctivitis Blepharitis Keratitis	Inflammation of the conjunctivae (exclude infectious causes and excluding uveitis as cause of red eye, also exclude conjunctivitis sicca which should not be scored as this is not a feature of active vasculitis); (specialist opinion not usually required). Inflammation of eyelids. Exclude other causes (trauma, infection). Usually no specialist opinion is required. Inflammation of central or peripheral cornea as evaluated by specialist.	1	1
Blurred vision	Altered measurement of best visual acuity from previous or baseline, requiring specialist opinion for further evaluation.	2	3
Sudden visual loss	Sudden loss of vision requiring ophthalmological assessment.		6
Uveitis	Inflammation of the uvea (iris, ciliary body, choroid) confirmed by ophthalmologist	2	6
Retinal vasculitis	Retinal vessel sheathing on examination by specialist or confirmed by retinal fluorescein angiography.	2	6
Retinal vessel thrombosis	Arterial or venous retinal blood vessel occlusion.		
Retinal exudates	Any area of soft retinal exudates (exclude hard exudates) seen on ophthalmoscopic examination.		
Retinal haemorrhages	Any area of retinal haemorrhage seen on ophthalmoscopic examination.		
4. ENT	**Maximum scores**	**3**	**6**

Nasal discharge/crusts/ulcers/granuloma	Bloody, mucopurulent, nasal secretion, light or dark brown crusts frequently obstructing the nose, nasal ulcers and/or granulomatous lesions observed by rhinoscopy.	2	4
Paranasal sinus involvement	Tenderness or pain over paranasal sinuses usually with pathologic imaging (CT, MR, x- ray, ultrasound).	1	2
Subglottic stenosis	Stridor and hoarseness due to inflammation and narrowing of the subglottic area observed by laryngoscopy.	3	5
Conductive hearing loss	Hearing loss due to middle ear involvement confirmed by otoscopy and/or tuning fork examination and/or audiometry.	1	3
Sensorineural hearing loss	Hearing loss due to auditory nerve or cochlear damage confirmed by audiometry.	2	6
5. Chest	**Maximum scores**	**3**	**6**
Wheeze or expiratory dyspnoea	Clinical signs of bronchial obstruction on examination.	1	2
Endobronchial/endotracheal involvement	Endobronchial pseudotumor or ulcerative lesions. Other causes such as infection or malignancy should be excluded. NB: smooth stenotic lesions to be included in VDI; subglottic lesions to be recorded in the ENT section.	2	4
Nodules or cavities	New lesions, detected by CXR.		3
Pleural effusion/pleurisy	Pleural pain and/or friction rub on clinical assessment or new onset of radiologically confirmed pleural effusion. Other causes (e.g. infection, malignancy) should be excluded.	2	4
Infiltrate	Detected by CXR or CT scan. Other causes (infection) should be excluded.	2	4
Massive haemoptysis/alveolar haemorrhage	Major pulmonary bleeding, with shifting pulmonary infiltrates; other causes of bleeding should be excluded if possible.	4	6
Respiratory failure	Dyspnoea which is sufficiently severe as to require artificial ventilation.	4	6
6. Cardiovascular	**Maximum scores**	**3**	**6**
Loss of pulses	Loss of pulses in any vessel detected clinically; this may include loss of pulses leading to threatened loss of limb.	1	4
Bruits over accessible arteries	Audible murmurs on auscultation or palpable bruits/thrills over large arteries and aorta.	1	2
Blood pressure discrepancy	>10 mm Hg difference in any limb.	1	2
Claudication of extremities	Focal muscle pain elicited usually by physical activity.	1	2
Ischaemic cardiac pain	Typical clinical history of cardiac pain leading to myocardial infarction or angina.	2	4
Cardiomyopathy	Significant impairment of cardiac function due to poor ventricular wall motion confirmed on echocardiography.	3	6
Congestive cardiac failure	Heart failure by history or clinical examination.	3	6
Valvular heart disease	Significant valve abnormalities in the aortic mitral or pulmonary valves detected clinically or echocardiographically.	2	4
Pericarditis	Pericardial pain &/or friction rub on clinical assessment.	1	3
7. Abdominal	**Maximum scores**	**5**	**9**
Abdominal pain	Persistent or recurrent abdominal pain, other than vasculitic causes excluded.	2	4
Peritonitis	Acute abdominal pain with peritonism/peritonitis due to perforation/infarction of small bowel, appendix or gallbladder etc., or acute pancreatitis confirmed by radiology/surgery/elevated amylase.	3	9

Blood in stools or bloody diarrhoea	Overt or occult blood in stools or bloody diarrhoea of recent onset; inflammatory bowel disease, anal fissure and infectious causes excluded.	2	6
Bowel ischaemic	Severe and recurrent abdominal pain often with GI bleeding due to ischaemic necrosis of the gut confirmed by imaging or at surgery, with typical appearances of aneurysms or abnormal vasculature characteristic of mesenteric vasculitis.	3	9
8. Renal	**Maximum scores**	**6**	**12**
Hypertension >95th centile (for height)	Systolic blood pressure greater than 95th centile by age and hight.	1	4
Proteinuria >0.3g/24h, >20mmol/mg creatinine	Persistent >20 mmol/mg creatinine and/or >0.3 g/24 hours.	2	4
Haematuria ≥2+ or 5 rbc/hpf or red cell casts	10 or more RBC per hpf (high power field), excluding urinary infection and urinary lithiasis (stone).	3	6
GFR 50-80ml/min/1.73m^2	Calculated or measured GFR 50-80ml/min/1.73m^2.	2	4
GFR 15-49ml/min/1.73m^2	Calculated or measured GFR 15-49ml/min/1.73m^2.	3	6
GFR <15ml/min/1.73m^2	Calculated or measured GFR <15ml/min/1.73m^2.	4	8
Rise in creatinine >10% or creatinine clearance (GFR) fall >25%	Significant deterioration in renal function attributable to active vasculitis. Rise in creatinine >10% when compared to previous value or fall in calculated or measured GFR >25%.		6
9. Nervous system	**Maximum scores**	**6**	**9**
Headache	New, unaccustomed & persistent headache.	1	1
Meningitis/encephalitis	Severe headache with neck stiffness ascribed to inflammatory meningitis after excluding infection/bleeding.	1	3
Organic confusion/cognitive dysfunction	Impaired orientation, memory or other intellectual function in the absence of metabolic, psychiatric, pharmacological or toxic causes.	1	3
Seizures (not hypertensive)	Focal motor, generalised or psychomotoric epileptic paroxysm, due to CNS vasculitis. Exclude idiopathic epilepsy, febrile seizures.	3	9
Stroke	Cerebrovascular accident resulting in focal neurological signs as paresis, weakness etc.	3	9
Cord lesion	Transverse myelitis with lower extremity weakness or sensory loss (usually with a detectable sensory level) with loss of sphincter control (rectal & urinary bladder).	3	9
Cranial nerve palsy	Facial nerve palsy, recurrent nerve palsy, oculomotor nerve palsy etc. excluding sensorineural hearing loss and ophthalmic symptoms due to inflammation.	3	6
Sensory peripheral neuropathy	Sensory neuropathy resulting in glove &/or stocking distribution of sensory loss. Other causes should be excluded (e.g., idiopathic, metabolic, vitamin deficiencies, infectious, toxic, hereditary).	3	6
Motor mononeuritis multiplex	Simultaneous neuritis of single or many peripheral nerves, only scored if motor involvement. Other causes should be excluded (diabetes, sarcoidosis, carcinoma, amyloidosis).	3	9
10. OTHER	Other feature of active vasculitis (e.g., malaise, pulmonary hypertension, auricular chondritis etc.) - please describe.		

Appendix 3 | The AXIS tool used for assessment of study quality in chapter 2.

AXIS Tool for Quality Appraisal	Yes	No	Don't know/comment
Introduction			
1. Were the aims/objectives of the study clear?			
Methods			
2. Was the study design appropriate for the stated aim(s)?			
3. Was the sample size justified?			
4. Was the target/reference population clearly defined? (Is it clear who the research was about?)			
5. Was the sample frame taken from an appropriate population base so that it closely represented the target/reference population under investigation?			
6. Was the selection process likely to select subjects/participants that were representative of the target/reference population under investigation?			
7. Were measures undertaken to address and categorise non-responders?			
8. Were the risk factor and outcome variables measured appropriate to the aims of the study?			
9. Were the risk factor and outcome variables measured correctly using instruments/measurements that had been trialled, piloted or published previously?			
10. Is it clear what was used to determined statistical significance and/or precision estimates? (e.g., p values, CIs)			
11. Were the methods (including statistical methods) sufficiently described to enable them to be repeated?			
Results			
12. Were the basic data adequately described?			
13. Does the response rate raise concerns about non-response bias?			
14. If appropriate, was information about non-responders described?			
15. Were the results internally consistent?			
16. Were the results for the analyses described in the methods, presented?			
Discussion			
17. Were the authors' discussions and conclusions justified by the results?			
18. Were the limitations of the study discussed?			
Other			
19. Were there any funding sources or conflicts of interest that may affect the authors' interpretation of the results?			
20. Was ethical approval or consent of participants attained?			

Appendix 4 | The data in collected from each paper included in the systematic review.

Abbreviations

Cr	Creatinine
eGFR	Estimated glomerular filtration rate
ELISA	Enzyme-linked immunosorbent assay
Ig	Immunoglobulin
IgAV	Immunoglobulin A vasculitis
IgAV-N	Immunoglobulin A vasculitis nephritis
IgAV-noN	Immunoglobulin A vasculitis without nephritis
IL	Interleukin
ISKDC	International Study of Kidney Disease in Children
KDIGO	Kidney Disease Improving Global Outcomes
PCR	Protein:creatinine ratio
UC	Urinary creatinine
UP	Urinary protein

Author	Year	Study design	Cohort demographic	Definition of nephritis	Type of sampling	Laboratory technique	Biomarker	Results
An et al. (46)	2018	Retrospective cross sectional	45 children with biopsy-confirmed IgAV-N grouped by pathological grade.	Renal histology, classified according to ISKDC.	24-hour urine collection	Turbidimetric method	Beta-2 microglobulin (β2-MG) Microalbumin (Malb) N-acetyl-beta-glucosaminidase (NAG) Transferrin (TfR)	Malb, TfR and NAG were different according to pathological grades (P<0.05). β2-MG was not statistically significantly increased.
Dyga et al. (48)	2020	Prospective longitudinal	11 paediatric patients IgAV-N (M=10, F=1) and 18 with IgAV-noN (M=7, F=11) compared to 34 healthy controls (M=23, F=11).	Haematuria: >5 erythrocytes per high power field ± UP/UC ratio >30mg/mmol ± eGFR <60 mL/min/1.73m^2.	One acute random urine sample and follow up sample 2-6 months after discharge	ELISA	Neutrophil gelatinase-associated lipocalin (NGAL) Kidney injury molecule-1 (KIM-1) Liver-fatty acid binding protein (L-FABP)	Acutely, all three biomarkers were increased in children with IgAV compared to controls (P <0.001), however not between the IgAV-N and IgAV-noN groups. At follow-up, NGAL was found to be increased in IgAV-N compared to IgAV-noN (P = 0.063).
Fang et al (49).	2020	Prospective cross sectional	30 children with IgAV-N (M=20, F=10) compared to 10 IgAV-noN (M=6, F=4) and 29 healthy controls (M=12, F=17).	Haematuria and/or proteinuria or renal biopsy results showing mesangial IgA deposition.	Midstream morning urine sample	ELISA	Integrin beta-1 (ITGB1) Tenascin	There were decreased urinary concentrations of both biomarkers in the IgAV-N cohort compared to controls (P <0.05). Tenascin was statistically significantly different in the IgAV-N vs IgAV-noN (P = 0.005).
Fuentes et al. (47)	2014	Prospective cross sectional	57 children had IgAV-N (M=32, F=25) and 20 with IgAV-noN (M=12, F=8), compared to 25 healthy volunteers (M=16, F=9).	Haematuria (>5 cells per high-power field in urine sediment) and/or proteinuria. Renal biopsy was classified using the ISKDC criteria.	First-morning urine sample	ELISA	Monocyte chemoattractant protein-1 (MCP-1)	Urinary MCP-1/Cr was increased in IgAV-N compared to the IgAV-noN and the controls (P <0.0001).

Ge et al. (60)	2014	Prospective longitudinal	34 paediatric patients with IgAV-noN (M=15, F=18), 37 with IgAV-N (M=18, F=19) and 37 healthy children (M=19, F=18).	Haematuria and/or proteinuria.	24-hour urine collection	ELISA	Microalbumin (Malb) Beta-2 microglobulin (β2-MG)	The concentrations were increased in IgAV-N patients compared to controls (*P* <0.05) and IgAV-noN (*P* <0.05).
Ma et al. (50)	2020	Prospective longitudinal	14 children with IgAV-N (M=7, F=7) vs 28 with IgAV-noN (M=16, F=12) and 23 healthy volunteers (M=9, F=14).	N/A[a]	Morning urine sample	N/A[a]	Urinary angiotensinogen (UAGT) Fibroblast specific protein-1 (FSP-1) Thrombin	UAGT and FSP-1 were increased in the IgAV-N cohort compared to controls and IgAV-noN (*P* <0.05). Thrombin was increased in all IgAV patients when compared to controls (*P* <0.05).
Mao et al. (53)	2012	Prospective longitudinal	51 paediatric patients with IgAV-noN (M=24, F=27) compared to 43 with haematuria but no proteinuria (M=21, F=22) and 13 with proteinuria (M=5, F=8).	Proteinuria (>1.0g/24h) and/or haematuria.	24-hour urine sample collected acutely and at follow up	ELISA	Urinary angiotensinogen (UAGT)	Acutely, UAGT concentrations were higher in those with proteinuria compared to IgAV-noN and IgAV with haematuria groups (*P* <0.0001). During the convalescent phase, UAGT concentrations were increased in the patients with proteinuria compared to IgAV-noN patients (*P* <0.0001) and the haematuria group (*P* <0.001).
Pillebout et al. (56)	2017	Prospective cross sectional	21 paediatric controls (M=13, F=8) were compared to 17 children with IgAV-noN (M=12, F=5) and 33 children with IgAV-N (M=20, F=13).	The presence of haematuria and/or a PCR >0.5 g/g and/or an eGFR <60 mL/min/1.73m^2.	N/A[b]	ELISA	IgA/Cr ratio (IgA/Cr) IgG/Cr ratio (IgG/Cr) IgM/Cr ratio (IgM/Cr) Igλ/IgK ratio (Igλ/IgK) IL-6/Cr ratio (IL-6/Cr) IL-8/Cr ratio (IL-8/Cr) IL-10/Cr ratio (IL10/Cr)	IgA/Cr and IgM/Cr were raised in IgAV-N compared to both controls and IgAV-noN (*P* <0.0001). IgG/Cr and the Igλ/IgK ratios were increased in IgAV-N compared to IgAV-noN (*P* <0.01). IL-6/Cr and IL-8/Cr were increased in IgAV-N compared to controls (*P* <0.0001) and IgAV-noN (*P*<0.01). IL-2/Cr was increased only when compared to IgAV-noN (*P*<0.01).
Qin et al. (61)	2011	Prospective cross sectional	68 children with IgAV-noN (M=33, F=35) were compared to 66 with IgAV-N (M=32, F=34) and 60 controls (M=29, F=31).	Patients categorized into normal concentrations of protein and haematuria; low-grade proteinuria (<1g/L) and/or haematuria; and heavy proteinuria (≥1g/L) and/or haematuria.	Mid-stream urine sample	ELISA	Matrix metalloproteinase-9 (MMP-9) Tissue inhibitor matrix metalloproteinase-1 (TIMP-1)	Urinary MMP-9, TIMP-1 and MMP-9/TIMP-1 were increased in IgAV-N compared to IgAV-noN (*P*<0.05) and controls (*P*<0.01). MMP-9 and MMP-9/TIMP-1 were increased in children with severe proteinuria compared to mild proteinuria (*P*<0.01) and moderate proteinuria (*P*<0.05).
Wang et al. (62)	2017	Prospective cross sectional	126 paediatric patients with IgAV-N (M=66, F=60) were compared to 135 non-nephritis IgAV children (M=71, F=64) and 84 healthy controls (M=48, F=36).	Haematuria and/or proteinuria within 6 months of the onset of rash. IgAV-N patients were further grouped into mild / moderate / severe proteinuria.	First-morning urine sample	ELISA	Monocyte chemoattractant protein-1 (MCP-1)	Urinary MCP-1 was increased in IgAV-N compared to controls and IgAV-noN (*P*<0.001). Concentrations also increased in parallel with the degree of proteinuria (all *P*<0.01).
Wang et al. (58)	2017	Prospective longitudinal	35 children (M=18, F=17) with IgAV-N, 41 paediatric patients (M=18, F=23) with a diagnosis of IgAV-noN and 32 healthy controls (M=17, F=15).	Haematuria and/or proteinuria within 6 months after the onset of rash.	Midstream first morning urine sample before and after treatment	ELISA	Macrophage migration inhibitory factor (MIF)	Urinary MIF was greatest in group I and higher than group II or controls (both *P*<0.05).

Ye et al. (57)	2015	Prospective cross sectional	694 children (M=332, F=362) with biopsy-proven IgAV-N, compared to 400 healthy controls (M=188, F=212).	Nephritis was graded according to the KDIGO criteria. Biopsy was classified according the ISKDC criteria.	N/A[b]	Roche Modular P800 biochemical analyser	24h urinary protein (24h-UPRO) Urinary protein:Cr ratio (U-PCR)	There was an increase in 24-UPRO and U-PCR when comparing those with grades I or IIa to grades IIb, IIIa or IIIb (*P*<0.01). 24-UPRO was increased in IgAV-N compared to controls (*P*<0.01).
Zhang et al. (55)	2015	Prospective longitudinal	27 children with IgAV-noN (M=19, F=8) were compared to 32 paediatric patients with IgAV-N (M=18, F=14) and 16 healthy volunteers (M=9, F=7).	Those who underwent a renal biopsy were graded according to ISKDC criteria. [c]	Spot morning urine samples	ELISA	Kidney injury molecule-1 (KIM-1) N-acetyl-beta-glucosaminidase (NAG) Beta-2 microglobulin (β2-MG)	Urinary KIM-1 concentrations were increased in IgAV-N compared to IgAV and controls (*P*<0.05). Patients with IgAV had an increased concentration of urinary KIM-1 compared to controls (*P*<0.001). NAG was highest in IgAV-N (*P*<0.05).

[a] As this study was not published in English, data was only extracted from the abstract and this information was not available.

[b] Method of urine sampling was not specified.

[c] Nephritis was not defined in this study.

Appendix 5 | Results of the study quality appraisal using the AXIS tool.

	An et al. (46)	**Dyga et al. (48)**	**Fang et al (49).**	**Fuentes et al. (47)**	**Ge et al. (60)**	**Ma et al. (50)**	**Mao et al. (53)**	**Pillebout et al. (56)**	**Qin et al. (61)**	**Wang et al. (62)**	**Wang et al. (58)**	**Ye et al. (57)**	**Zhang et al. (55)**
Introduction													
1. Were the aims/objectives of the study clear?	Y	Y	Y	Y	Y	N/A	Y	Y	Y	Y	Y	Y	Y
Methods													
2. Was the study design appropriate for the stated aim(s)?	Y	Y	Y	Y	Y	N/A	Y	Y	Y	Y	Y	Y	Y
3. Was the sample size justified?	N	Y	Y	Y	Y	N/A	Y	Y	Y	Y	Y	Y	Y
4. Was the target/reference population clearly defined? (Is it clear who the research was about?)	Y	Y	Y	Y	Y	N/A	Y	Y	Y	Y	Y	Y	Y
5. Was the sample frame taken from an appropriate population base so that it closely represented the target/reference population under investigation?	Y	Y	Y	Y	Y	N/A	Y	Y	Y	Y	Y	Y	Y
6. Was the selection process likely to select subjects/participants that were	Y	Y	Y	Y	Y	N/A	Y	Y	Y	Y	Y	Y	Y

representative of the target/reference population under investigation?													
7. Were measures undertaken to address and categorise non-responders?	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
8. Were the risk factor and outcome variables measured appropriate to the aims of the study?	DK	Y	Y	Y	Y	N/A	Y	Y	Y	Y	Y	Y	Y
9. Were the risk factor and outcome variables measured correctly using instruments/measurements that had been trialled, piloted or published previously?	Y	Y	Y	DK	Y	N/A	Y	Y	Y	Y	Y	Y	Y
10. Is it clear what was used to determined statistical significance and/or precision estimates? (e.g., p values, CIs)	Y	Y	Y	Y	Y	N/A	Y	Y	Y	Y	Y	Y	Y
11. Were the methods (including statistical methods) sufficiently described to enable them to be repeated?	Y	Y	Y	Y	Y	N/A	Y	Y	Y	Y	Y	Y	Y
Results													
12. Were the basic data adequately described?	N	Y	N	Y	Y	N/A	Y	Y	Y	Y	Y	Y	Y
13. Does the response rate raise concerns about non-response bias?	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
14. If appropriate, was information about non-responders described?	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
15. Were the results internally consistent?	Y	N	Y	Y	Y	N/A	Y	Y	Y	Y	Y	N	Y
16. Were the results for the analyses described in the methods, presented?	Y	Y	Y	Y	Y	N/A	Y	Y	Y	Y	Y	Y	Y
Discussion													
17. Were the authors' discussions and conclusions justified by the results?	Y	Y	Y	Y	Y	N/A	Y	Y	Y	Y	Y	Y	Y
18. Were the limitations of the study discussed?	Y	Y	Y	Y	Y	N/A	N	N	N	Y	Y	N	Y
Other													
19. Were there any funding sources or conflicts of interest that may affect the authors' interpretation of the results?	N	N	N	DK	N	N/A	DK	N	DK	N	DK	N	N
20. Was ethical approval or consent of participants attained?	Y	Y	Y	Y	Y	N/A	Y	Y	Y	Y	Y	Y	Y
Overall score													
	14/20	16/20	16/20	15/20	17/20	N/A	15/20	16/20	15/20	17/20	16/20	15/20	17/20

Appendix 6 | The frequency of biomarker identification in chapter 2.

Biomarker identified	Studies
Beta-2 microglobulin (β2-MG)	An et al. (46) Ge et al. (52) Qin et al. (51) Zhang et al. (55)
24h urinary protein (24h-UPRO)	Ye et al. (57)
Fibroblast specific protein-1 (FSP-1)	Ma et al. (50)
Immunoglobulin λ/Immunoglobulin K ratio (Igλ/IgK ratio)	Pillebout et al. (56)
Immunoglobulin A/Cr ratio (IgA/Cr) [a]	Pillebout et al. (56)
Immunoglobulin G/Cr ratio (IgG/Cr) [a]	Pillebout et al. (56)
Immunoglobulin M/Cr ratio (IgM/Cr) [a]	Pillebout et al. (56)
Interleukin-6/Cr ratio (IL-6/Cr) [a]	Pillebout et al. (56)
Interleukin-8/Cr ratio (IL-8/Cr) [a]	Pillebout et al. (56)
Interleukin-10/Cr ratio (IL10/Cr) [a]	Pillebout et al. (56)
Integrin beta-1 (ITGB1)	Fang et al. (49)
Kidney injury molecule-1 (KIM-1)	Dyga et al. (48) Zhang et al. (55)
Liver-fatty acid binding protein (L-FABP)	Dyga et al. (48)
Urinary albumin concentration (Malb)	An et al. (46) Ge et al. (52)
Monocyte chemoattractant protein-1 (MCP-1)	Fuentes et al. (47) Wang et al. (58)
Macrophage migration inhibitory factor (MIF)	Wang et al. (54)
Matrix metalloproteinase-9 (MMP-9)	Qin et al. (51)
N-acetyl-beta-glucosaminidase (NAG)	An et al. (46) Zhang et al. (55)
Neutrophil gelatinase-associated lipocalin (NGAL)	Dyga et al. (48)
Transferrin (TfR)	An et al. (46)
Tissue inhibitor matrix metalloproteinase-1 (TIMP-1)	Qin et al. (51)
Urinary angiotensinogen (UAGT)	Ma et al. (50) Mao et al. (50)
Urinary protein:Cr ratio (U-PCR) [a]	Ye et al. (57)

[a] *Cr refers to creatinine*

Appendix 7 | The original IgA-VAS scoring tool distributed to clinicians in January 2020.

CUTANEOUS

None _____0 [ ]
Petechial and/or purpuric rash _____0 [ ]
Skin blistering _____0 [ ]
Ulceration _____0 [ ]
Necrotic areas _____0 [ ]
Vasculitic gangrene _____0 [ ]

GASTROINTESTINAL

None _____0 [ ]
Ischaemic abdominal pain manageable with simple analgesia _____0 [ ]
Ischaemic abdominal pain requiring strong analgesia _____0 [ ]
Vomiting _____0 [ ]
Diarrhoea _____0 [ ]
Blood in stools _____0 [ ]
Intussusception _____0 [ ]

MUSCULOSKELETAL

None _____0 [ ]
Malaise/lethargy _____0 [ ]
Arthralgia _____0 [ ]
Arthritis _____0 [ ]
Myalgia _____0 [ ]

RENAL

None _____0 [ ]
Proteinuria >1+ on dipstick _____0 [ ]
Proteinuria with a urine PCR >250mg/mmol Cr (or equivalent) _____0 [ ]
Haematuria >1+ on dipstick _____0 [ ]
Gross haematuria _____0 [ ]
Hypertension (taken as 3 readings >95th centile for child's age, sex and height) _____0 [ ]
Nephrotic syndrome (oedema, low serum albumin, heavy proteinuria) _____0 [ ]
Rise in creatinine above baseline value (or upper limit of normal for age range) _____0 [ ]
Rise in creatinine >1.5x above baseline value (or upper limit of normal for age range) _____0 [ ]

Scoring

Domains

- Cutaneous score (max.)
- Abdominal score (max.)
- Musculoskeletal score (max.)
- Renal score (max.)

Total score = (max.)

Appendix 8 | The Medical Research Council decision on whether ethical approval is needed.

Go straight to content.

Medical Research Council

NHS Health Research Authority

Is my study research?

To print your result with title and IRAS Project ID please enter your details below:

Title of your research:

The development and preliminary validation of the IgA-VAS scoring tool

IRAS Project ID (if available):

You selected:

- **'No'** - Are the participants in your study randomised to different groups?
- **'No'** - Does your study protocol demand changing treatment/ patient care from accepted standards for any of the patients involved?
- **'No'** - Are your findings going to be generalisable?

Your study would NOT be considered Research by the NHS.

You may still need other approvals.

Researchers requiring further advice (e.g. those not confident with the outcome of this tool) should contact their R&D office or sponsor in the first instance, or the HRA to discuss your study. If contacting the HRA for advice, do this by sending an outline of the project (maximum one page), summarising its purpose, methodology, type of participant and planned location as well as a copy of this results page and a summary of the aspects of the decision(s) that you need further advice on to the HRA Queries Line at Queries@hra.nhs.uk.

For more information please visit the Defining Research table.

Follow this link to start again.

Print This Page

NOTE: If using Internet Explorer please use browser print function.

About this tool Feedback Contact Glossary Accessibility

Appendix 9 | The final IgA-VAS scoring tool piloted retrospectively on children who were admitted to or were seen in clinic at Alder Hey Children's Hospital between 01 January 2017 and 31 December 2019.

CUTANEOUS INVOLVEMENT – max 24	
None	0
Distribution most common - legs, arms, buttocks	1
Distribution common trunk, chest, feet	1
Distribution uncommon palms	2
Distribution rare face, head, neck	3
Petechial and/or purpuric rash	1
Skin blistering	3
Ulceration	4
Necrotic areas	4
Vasculitic gangrene	5

GASTROINTESTINAL INVOLVEMENT – max 19	
None	0
Ischaemic abdominal pain manageable with analgesia from step 1 of the WHO analgesic ladder (non-opioid analgesics and NSAIDs +/- adjuvants)	1
Ischaemic abdominal pain requiring analgesia from step 2 of the WHO analgesic ladder (weak opioids +/- adjuvants)	2
Ischaemic abdominal pain requiring analgesia from step 3 of the WHO analgesic ladder (strong opioids +/- adjuvants)	3
Intermittent vomiting but tolerating oral diet	1
Severe vomiting and not tolerating oral diet	2
Diarrhoea	1
Melaena or gastrointestinal bleeding	4
Intussusception	5

MUSCULOSKELETAL INVOLVEMENT – max 5	
None	0
Malaise/lethargy	1
Arthralgia	1
Myalgia	1
Arthritis	2

RENAL INVOLVEMENT – max 52	
None	0
Microscopic haematuria	1
Gross haematuria	2
Hypertension (taken as 3 reading >9th centile for child's age, sex and height)	2
Proteinuria >1+ on dipstick	2
Proteinuria with a urine PCR >250mg/mmol Cr (or equivalent)	3
Persistent proteinuria (2+ or more) beyond 3 months	3
Nephrotic syndrome (oedema, low serum albumin, heavy proteinuria)	5
Estimated GFR 50-80 ml/min/1.73m^2	6
Estimated GFR 15-49 ml/min/1.73m^2	8

Estimated GFR <15 ml/min/1.73m^2	10
Histological evidence of IgAV-nephritis	10

OTHER MANIFESTATIONS – max 25	
Constitutional features (fever, weight loss, lymphadenopathy)	2
Orchiditis (such as scrotal pain or swelling)	3
Pulmonary haemorrhage	10
Neurological involvement (headaches, encephalitis or seizures)	10

Total score

Domains

CUTANEOUS	/24
GASTROINTESTINAL	/19
MUSCULOSKELETAL	/5
RENAL	/52
OTHER	/25

TOTAL SCORE = /125

Appendix 10 | The updated IgA-VAS created following the preliminary validation. This tool is now ready for prospective validation to assess inter-rater reliability.

IgA-VAS

Purpose for use: This tool aims to score the disease activity of children with a diagnosis of IgA vasculitis.
Instructions for use: Any disease features identified should be present within the 4 weeks previous and have other causes excluded. Where there are different severities of the same manifestation, all boxes should be ticked, e.g., if a child has macroscopic haematuria, they should score for both microscopic and macroscopic haematuria. Where possible, urine samples should be an early morning sample. For some features, e.g., urine protein:creatinine ratio (PCR), conversions may be required to suit local assays.

CUTANEOUS INVOLVEMENT – max 24 (tick all that apply)	
None	0
Distribution	
Most common - legs, arms, buttocks	1
Common - trunk, chest, feet	1
Uncommon - palms	2
Rare – including face, head, neck	3
Characteristic	
Petechial and/or purpuric rash	1
Skin blistering	3
Ulceration	4
Necrotic areas	4
Vasculitic gangrene	5

GASTROINTESTINAL INVOLVEMENT – max 19 (tick all that apply)	
None	0
Ischaemic abdominal pain manageable with analgesia from step 1 of the WHO analgesic ladder (non-opioid analgesics and NSAIDs +/- adjuvants)	1
Ischaemic abdominal pain requiring analgesia from step 2 of the WHO analgesic ladder (weak opioids +/- adjuvants)	2
Ischaemic abdominal pain requiring analgesia from step 3 of the WHO analgesic ladder (strong opioids +/- adjuvants)	3
Intermittent vomiting but tolerating oral diet	1
Severe vomiting and not tolerating oral diet	2
Diarrhoea	1
Melaena or gastrointestinal bleeding	4
Intussusception or features on endoscopy e.g., intramural bleeding	5

MUSCULOSKELETAL INVOLVEMENT – max 5 (tick all that apply)	
None	0
Malaise/lethargy	1
Arthralgia	1
Myalgia	1
Arthritis	2

RENAL INVOLVEMENT – max 52 (tick all that apply)	
None	0
Microscopic haematuria (>1+ on dipstick in the absence of macroscopic haematuria)	1
Macroscopic haematuria	2
Hypertension (taken as 3 reading >9th centile for child's age, sex and height)	2
Mild-moderate proteinuria (>1+ on dipstick with a urine PCR<250mg/mmol or equivalent)	2
Moderate-severe proteinuria (>1+ on dipstick with a urine PCR >250mg/mmol or equivalent)	3
Persistent proteinuria (2+ or more) beyond 3 months from diagnosis	3
Nephrotic syndrome (oedema, low serum albumin, heavy proteinuria)	5
Estimated GFR 50-80 ml/min/1.73m^2	6
Estimated GFR 15-49 ml/min/1.73m^2	8
Estimated GFR <15 ml/min/1.73m^2	10
Histological evidence of IgAV-nephritis	10

OTHER MANIFESTATIONS – max 25 (tick all that apply)	
Constitutional features (fever in the absence of infection, weight loss, lymphadenopathy)	2
Orchiditis (such as scrotal pain or swelling)	3
Pulmonary haemorrhage	10
Neurological involvement (headaches, encephalitis or seizures)	10

Total score

Domains

CUTANEOUS	/24
GASTROINTESTINAL	/19
MUSCULOSKELETAL	/5
RENAL	/52
OTHER	/25

TOTAL SCORE = /125

Abbreviations

GFR – glomerular filtration rate

NSAIDs – non-steroidal anti-inflammatory drugs

PCR – protein:creatinine ratio

WHO – World Health Organisation