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# Variations in Urinary Renin-Angiotensin-Aldosterone Component Concentrations in Paediatric IgA Vasculitis Nephritis

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**Abstract:** IgA Vasculitis (IgAV) is the most common form of vasculitis in children, and 1–2% of patients develop chronic kidney disease. In other forms of glomerulonephritis, there is strong evidence to support the role of the renin-angiotensin-aldosterone system (RAAS); however, data are lacking in IgAV nephritis. This study evaluated urinary RAAS components in children with IgA vasculitis, both with nephritis (IgAVN) and without nephritis (IgAVwoN). Urinary concentrations of renin, angiotensinogen and aldosterone were quantified using ELISAs. In total, 40 patients were included: IgAVN n = 9, IgAVwoN n = 17, HC n = 14, with a mean age of  $8.3 \pm 3.3$  years. Urinary renin demonstrated no trend with nephritis. Urinary angiotensinogen was statistically significantly elevated in IgAV ( $1.18 \pm 1.16$  ng/mmol) compared to HC ( $0.28 \pm 0.27$  ng/mmol,  $p = 0.0015$ ), and IgAVN ( $2.00 \pm 1.22$  ng/mmol) was elevated compared to IgAVwoN ( $0.74 \pm 0.89$  ng/mmol,  $p = 0.0492$ ) and HC ( $p = 0.0233$ ). Urinary aldosterone levels were significantly elevated in IgAV ( $1236 \pm 1438$  pg/mmol) compared to HC ( $73.90 \pm 65.22$  pg/mmol,  $p < 0.0001$ ); this was most increased in IgAVwoN patients ( $1793 \pm 1507$  pg/mmol; IgAVN  $183.30 \pm 111.30$  pg/mmol,  $p = 0.0035$ , HC  $p < 0.0001$ ). As expected, the RAAS system is activated in patients with IgAVN and, more surprisingly, even in those without active nephritis. Further studies are needed to fully understand the role of the RAAS system in IgA vasculitis.

**Keywords:** Henoch-Schoenlein purpura; RAAS; renal; children; paediatric; urine



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## 1. Introduction

Immunoglobulin A Vasculitis (IgAV), formerly known as Henoch-Schönlein purpura [1], is the most common form of vasculitis in childhood [2,3]. It is diagnosed at a rate of 3–27 per 100,000 children per year [3]. While it can present at any age, 90% of cases are diagnosed before the age of 10 years old, with a peak incidence between 4 and 6 years of age [2]. IgAV typically presents with a palpable purpuric non-blanching rash, predominantly localised to the lower limbs and associated with at least one of the following other symptoms: abdominal pain, joint pain and/or nephritis [4]. In the majority of cases, IgAV is self-limiting and resolves with minimal intervention over a few weeks [2]. However, around 40% of patients will develop renal inflammation, known as IgAV-nephritis (IgAVN), which is the most serious clinical manifestation of IgAV in terms of long-term morbidity and mortality. In total, 1–2% of all patients goes on to develop chronic kidney disease (CKD) stage 5 [2,5]. Early stratification of the patients at high risk of disease progression is essential, and understanding the pathophysiology of IgAVN may allow for earlier intervention [2].

Angiotensinogen (AGT), produced by the liver, is cleaved by renin to form angiotensin 1. This is further shortened to angiotensin II, the main effector of the renin-angiotensin-aldosterone system (RAAS), via the angiotensin converting enzyme 1 (ACE). Angiotensin II acts upon the adrenal zona glomerulosa of the adrenal gland, regulating the secretion of aldosterone. The RAAS pathway is vital for homeostatic control of blood pressure, volume and electrolyte levels through systematic effects on the heart, vasculature and kidneys [6,7]. Angiotensin II drives vasoconstriction on the primary post-glomerular arterioles, impacting upon glomerular hydraulic pressure, protein ultrafiltration and the secretion of aldosterone.

ACE inhibitors (ACEi), angiotensin receptor blockers and aldosterone antagonists have been used for their renoprotective effects by slowing or halting the progression of CKD in a variety of glomerular diseases, including those in the paediatric population [6,8,9]. A recent protein array analysis of urinary proteins, carried out in a small patient cohort with IgAV, suggested RAAS as a pathway of significant interest [10], where angiotensinogen was elevated and renin was unchanged [10–13]. This study aimed to evaluate urinary RAAS components in children with IgA vasculitis nephritis.

## 2. Materials and Methods

### 2.1. Patient Selection and Definitions

The patients were selected using those recruited to the IgA Vasculitis study, a single-centre observational study at Alder Hey Children's NHS Foundation Trust, Liverpool, UK, between 28 August 2019 and 6 May 2022. Patients of any gender who were aged <18 years old at their first presentation of IgAV were included. The diagnosis was made in line with the EULAR/PRINTO/PReS 2008 criteria [4]. The participant exclusion criteria were as follows: (A) patients with an uncertain diagnosis of IgAV; (B) patients with another concurrent inflammatory or renal condition; and/or (C) patients undergoing dialysis.

Participants were grouped into IgAV and healthy controls (HC), and those with IgAV were further divided according to the presence of nephritis (IgAVN) or without nephritis (IgAVwoN). IgAVN was defined as a urinary albumin to creatinine ratio (UACR) of >30 mg/mmol at the time of urine sampling [4]. The HC participants were age and sex-matched, and consisted of children aged <18 years old with no past medical history of autoimmune or renal disease and those who were not taking any regular medication for other conditions. The HC participants were recruited from patients attending for day-case investigations or day-case surgery. There was no minimum follow-up required for inclusion; however, for the participants included in the IgAV without active nephritis (IgAVwoN) group, a sample was obtained within 4 weeks of diagnosis. All urine samples were collected as a midstream spot urine sample.

### 2.2. Data Collection

Demographic and clinical data were collected at the time of sampling for baseline characteristics. This included sex, age, ethnicity, blood pressure (BP), height, serum creatinine, serum albumin (where available), UACR, renal histology report and any medication. The renal histology (in cases where a renal biopsy was conducted) was graded according to the International Study of Kidney Disease in Children classification for IgAVN [14]. The participants BP was defined as hypertensive if the systolic BP was above the 95th centile for the child's age, sex and height for those participants aged <16 years old, or a systolic BP > 140 mmHg for children aged 16 years and older [15]. In cases with missing height data, the value corresponding to the 50th centile for the participants age was used to improvise where height data was not available within 2 months of the BP reading. The age-specific laboratory reference ranges used at Alder Hey Children's NHS Foundation Trust (Liverpool, UK) were used to define out-of-range blood results. At the last medical review, renal outcome, UACR and medication were recorded. For the IgAVN patients, full remission was defined as a UACR < 30 mg/mmol, partial remission was defined as a UACR that had any improvement from baseline but remained >30 mg/mmol and no response/worsening disease was defined as a UACR that was greater than the baseline

result. A UACR of 0 mg/mmol was assumed for patients with a urine dipstick that was negative for any protein.

### 2.3. Sample Processing

Healthy control urine samples were discarded if a urine dipstick test was positive for leukocytes, nitrites, blood or >+1 for protein. Urine was centrifuged twice at  $300\times g$  for 10 min before being frozen for storage at  $-80\text{ }^{\circ}\text{C}$ . Plasma was isolated from 1 mL heparinised blood and centrifuged twice at  $300\times g$  for 10 min, then the plasma was stored at  $-80\text{ }^{\circ}\text{C}$ . Samples were defrosted on the day of analysis and vortexed for 10 s.

### 2.4. ELISA Assay

To quantify the renin and aldosterone, commercially available enzyme-linked immunosorbent assays (ELISA) kits were purchased from Bio-Techne (Abingdon, UK) and angiotensinogen ELISA was purchased from Life Technologies Limited (Paisley, UK). All urine samples were run neat, following the manufacturer's protocols. In the case of renin, the plasma samples were run following ten-fold dilution.

#### 2.4.1. Renin

For the renin assay, 100  $\mu\text{L}$  of assay diluent and 50  $\mu\text{L}$  of sample or standard were added into each well, incubated for 2 h at  $36\text{ }^{\circ}\text{C}$  and agitated at 500 rpm. The plate was washed, and 200  $\mu\text{L}$  renin conjugate was added and incubated for 2 h. The wells were washed again, loaded with 200  $\mu\text{L}$  substrate solution and incubated for 30 min. Then, 50  $\mu\text{L}$  stop solution was added and results were measured.

#### 2.4.2. Angiotensinogen

For the angiotensinogen assay, 100  $\mu\text{L}$  of standard or sample was aliquoted into each well and incubated at  $36\text{ }^{\circ}\text{C}$  for 2.5 h whilst mixing at 500 rpm. The wells were washed, and 100  $\mu\text{L}$  biotin conjugate was added and incubated for 1 h. After washing, 100  $\mu\text{L}$  streptavidin-HRP solution was loaded and incubated for 45 min. The plate was washed, then 100  $\mu\text{L}$  TMB substrate was added and incubated for 30 min before the addition of stop solution and measurement of the results.

#### 2.4.3. Aldosterone

For this assay, the wells were coated with Aldosterone primary antibody and allowed to incubate for 1 h at  $36\text{ }^{\circ}\text{C}$ , while mixing at 500 rpm. The wells were washed and loaded with 50  $\mu\text{L}$  assay or calibrant diluent, then 75  $\mu\text{L}$  of sample/standard was loaded, followed by 50  $\mu\text{L}$  aldosterone conjugate, and incubated for 2 h. Wells were washed, and then 200  $\mu\text{L}$  of substrate solution was added and incubated for 30 min. Finally, 100  $\mu\text{L}$  of stop solution was added before data recording.

All ELISAs were read using a POLARstar Omega plate reader (BMG LABTECH GmbH, Ortenberg, Germany), measuring at 450 nm, with subtracting noise that was measured at 570 nm. Calibration curves were constructed and sample concentrations were determined using MARS Data Analysis Software version 3.32 (BMG LABTECH GmbH).

### 2.5. Urinary Creatinine Quantification

In order to normalise for varying urine concentrations, all concentrations were divided by the urinary creatinine concentration for each patient. Automated quantification of urinary creatinine was performed using a previously described enzymatic method [16] on an Abbott Architect Ci8200 (Abbott, Chicago, IL, USA).

### 2.6. Ethical Approval

All experiments were conducted in accordance with the Declaration of Helsinki and the Human Tissue Act. The work was carried out under the IgA Vasculitis study, which was approved by the HRA and Health and Care Research Wales on 21 June 2019 (REC

17/NE/0390, IRAS 236599). Written informed consent was obtained from parents and children before sample collection.

### 2.7. Statistical Analysis

Statistical analysis was performed using GraphPad Prism version 8.0 (GraphPad Software, San Diego, CA, USA). Due to the relatively small sample size in some of the renal status groups, it was assumed all data were non-normally distributed and evaluated using a Mann–Whitney U test or a Kruskal–Wallis with a Dunn–Bonferroni post hoc test. Pearson's correlation coefficient ( $r$ ) was used to assess for correlation between urinary RAAS components and proteinuria (UACR) in the patients with IgAV. A negligible coefficient is deemed to be  $<0.30$ , with  $0.30$ – $0.50$  considered a low correlation,  $0.50$ – $0.70$  a moderate correlation,  $0.70$ – $0.90$  a high correlation and  $0.90$ – $1.00$  a very high correlation [17]. A  $p$ -value of  $<0.05$  was considered statistically significant. All data are reported as mean plus/minus standard deviation and the number of samples in each group during analysis. When samples returned a value too low for detection, they were excluded from the statistical analysis.

## 3. Results

### 3.1. Paediatric Cohort

In total, 40 children were recruited in this study, which consisted of 26 children with IgAV (IgAVN  $n = 9$ , IgAVwoN  $n = 17$ , HC  $n = 14$ ); their baseline characteristics are presented in Table 1. The mean age of the cohort was  $8.3 \pm 3.3$  years old, 58% of the cohort were male and 80% were Caucasian. Children with IgAVwoN were significantly younger ( $6.7 \pm 3.0$  years old) than patients in the HC group ( $10.3 \pm 2.7$  years old;  $p = 0.04$ ). At baseline, three patients with IgAVN had hypertension compared to one IgAVwoN. Two patients had elevated serum creatinine levels outside of their age-specific reference range (IgAVN,  $n = 1$ ; IgAVwoN,  $n = 1$ , range 75–85 mg/dL), whilst four had mildly reduced serum albumin levels (IgAVN,  $n = 3$ ; IgAVwoN,  $n = 1$ ; range 32–39 g/L). No patients had any evidence of peripheral oedema. At the time of sample collection, no patients were on any immunosuppressive medications or RAAS inhibitors.

Within the IgAVN group, four patients had a renal biopsy demonstrating IgA predominant deposits on immunofluorescence. C3 positivity was present in all four patients: IgG in two and fibrinogen in one.

The mean follow-up was  $11.4 \pm 17.1$  months. At the last review, none of the IgAVwoN patients progressed onto IgAVN during follow up, and amongst IgAVN, five were in partial remission and four in full remission. Of these IgAVN patients, two were on corticosteroids, two on mycophenolate mofetil and two on an ACEi (lisinopril) at the time of the last clinical review.

**Table 1.** Patients characteristics at baseline. <sup>a</sup>  $n$  (%); <sup>b</sup> mean  $\pm$  SD. <sup>1</sup> BP readings available for 8 patients of the IgAVwoN group. <sup>2</sup> Serum creatinine was available for 13 patients (IgAVN,  $n = 8$ ; IgAVwoN,  $n = 5$ ). <sup>3</sup> Serum albumin was available for 11 patients (IgAV,  $n = 7$ ; IgAVwoN,  $n = 4$ ). UACR: urinary albumin to creatinine ratio. ISKDC: International Study for Kidney Disease in Children Classification. Significant  $p$ -values are highlighted in bold. +  $p < 0.05$  compared to IgAVwoN. Due to rounding, percentages may not add up to 100.

	Overall	IgAVN	IgAVwoN	HC	$p$ -Value
$n$ (%)	40 (100%)	9 (23%)	17 (43%)	14 (35%)	-
Male <sup>a</sup>	23 (28%)	6 (67%)	10 (59%)	7 (50%)	0.725
Age, years <sup>b</sup>	$8.3 \pm 3.3$	$8.3 \pm 3.5$	$6.7 \pm 3.0$	$10.3 \pm 2.7$ <sup>+</sup>	0.006
Ethnicity					
British	32 (80%)	7 (79%)	15 (88%)	10 (71%)	
African	3 (8%)	0 (0%)	0 (0%)	3 (21%)	
Chinese	2 (5%)	0 (0%)	2 (12%)	0 (0%)	
Any other Asian background	1 (3%)	0 (0%)	0 (0%)	1 (7%)	
Not stated	2 (5%)	2 (22%)	0 (0%)	0 (0%)	

Table 1. Cont.

	Overall	IgAVN	IgAVwoN	HC	p-Value
Sampling					
Sample type					
Urine and blood <sup>a</sup>	19 (100%)	5 (26%)	5 (26%)	9 (47%)	
Urine only <sup>a</sup>	26 (100%)	3 (12%)	18 (69%)	5 (19%)	
Blood only <sup>a</sup>	5 (100%)	5 (100%)	-	-	
Weeks from diagnosis to sampling <sup>b</sup>	18.4 ± 60.0	49.7 ± 97.8	1.8 ± 0.9	-	<0.001
Renal involvement					
Hypertension <sup>a,1</sup>	4 (23.5%)	3 (33.3%)	1 (12.5%)	-	0.312
Serum creatinine mg/dL <sup>b,2</sup>	51.5 ± 18.5	53.3 ± 17.2	48.6 ± 22.1	-	0.769
Serum albumin g/L <sup>b,3</sup>	39.3 ± 4.3	37.1 ± 3.1	43.0 ± 3.7	-	0.029
UACR mmol/mg <sup>b</sup>	90.7 ± 147.6	257.8 ± 141.2	2.2 ± 5.5	-	<0.001
Urinary creatinine mmol/L <sup>b</sup>	8.7 ± 7.6	6.2 ± 4.7	7.7 ± 7.7	11.5 ± 8.4	0.146
Biopsy proven nephritis <sup>a</sup>		4 (44%)			
ISKDC Grade					
IIIa <sup>a</sup>	-	1 (25%)	-	-	-
IIIb <sup>a</sup>	-	2 (50%)	-	-	-
IV <sup>a</sup>	-	1 (25%)	-	-	-
Medications					
Corticosteroids <sup>a</sup>		2			
Follow-up					
Months <sup>b</sup>	11.4 ± 17.1	23.2 ± 25.0	5.1 ± 5.2	-	0.005
UACR at last review <sup>b</sup>	10.9 ± 20.5	31.6 ± 24.0	0.0 ± 0.0	-	<0.001
Outcome <sup>a</sup>		Full remission, 4 (44%) Partial remission, 5 (56%) Worsened, 0 (0%)			

### 3.2. Urinary and Plasma Renin Concentration

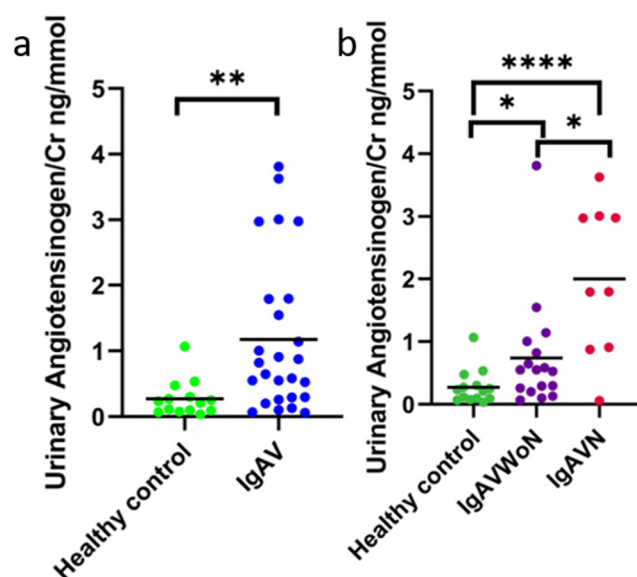
Urine renin concentrations were below the level of detection in the majority of samples, i.e., 6 HC (43%), 12 IgAVwoN (71%) and 6 IgAVN (67%) of the samples. No trends were discernible between patient groups; however, the sample size where renin was detected was too small for reliable statistical analysis.

Due to the low urinary concentration of renin, plasma renin was analysed in a subset of HC, IgAVwoN and IgAVN participants (selected participants from those where plasma was available). No significant differences were observed in the plasma renin concentrations in the HC ( $169.60 \pm 56.19$  pg/mL,  $n = 9$ ) compared to all IgAV participants ( $254.90 \pm 146.50$  pg/mL,  $n = 14$ ;  $p = 0.1093$ ). However, the plasma renin concentration was increased between IgAVN ( $2825 \pm 1644$  pg/mL,  $n = 10$ ) and IgAVwoN ( $1857 \pm 540.60$  pg/mL,  $n = 4$ ), although the number of samples available did not allow for reliable statistical analysis to be performed.

### 3.3. Urinary Angiotensinogen Concentration

Urinary angiotensinogen (AGT) was detected in all of the samples. The urinary concentrations of AGT/Cr in all participants with IgAV ( $1.18 \pm 1.16$  ng/mmol,  $n = 26$ ) were statistically significantly increased compared to HC ( $0.28 \pm 0.27$  ng/mmol,  $n = 14$ ;  $p = 0.0015$ ) (Figure 1a). When grouping patients according to the presence of nephritis, the urinary AGT/Cr concentrations were statistically significantly increased by 7.2-fold in the IgAVN group ( $2.00 \pm 1.22$  ng/mmol,  $n = 9$ ) compared to HC ( $p = 0.0006$ ) (Figure 1b). Similarly, the urinary AGT/Cr concentrations were greater in the IgAVwoN patients ( $0.74 \pm 0.89$  ng/mmol,  $n = 17$ ) when compared to the HC participants ( $p = 0.0233$ ). There were also statistically significantly increased urinary AGT/Cr concentrations in the patients with IgAVN compared to the IgAVwoN group (2.7-fold,  $p = 0.0492$ ). An obvious outlier was noted within the IgAVwoN group (urinary AGT/Cr 1.8 ng/mmol), as illustrated.

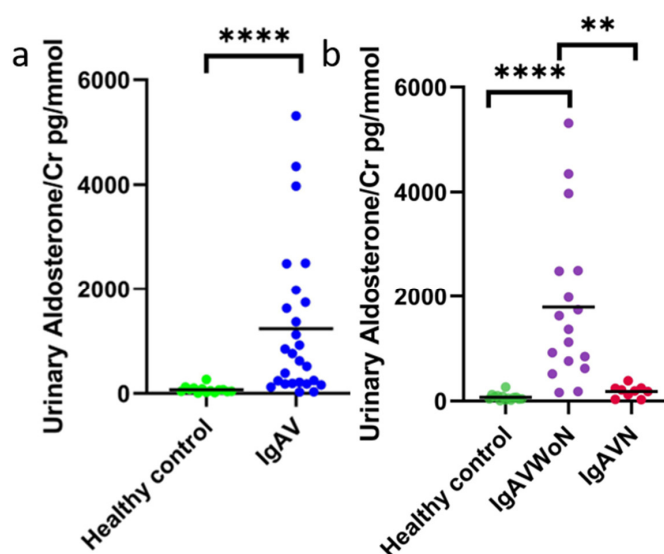




**Figure 1.** Urinary creatinine normalised angiotensinogen concentration, separated by disease and renal involvement status. The mean concentration for each patient group is denoted. (a) Urinary angiotensinogen is significantly elevated in IgAV patients compared to HC. (b) When separated by renal involvement, angiotensinogen levels were elevated compared to HC regardless of the presence of renal disease; which was defined as  $>30$  mg/mmol creatinine, with IgAVN having the greatest angiotensinogen concentration. \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*\*  $p < 0.0001$ .

### 3.4. Urinary Aldosterone Concentration

Urinary aldosterone was detected in all of the samples. The concentration of urinary aldosterone/Cr in all IgAV patients ( $1236 \pm 1438$  pg/mmol,  $n = 26$ ) was statistically significantly increased compared to HC ( $73.90 \pm 65.22$  pg/mmol,  $n = 14$ ;  $p < 0.0001$ ) (Figure 2a). When patients were divided according to the presence of nephritis, surprisingly, the IgAVwoN had a statistically significantly increased urinary aldosterone/Cr concentration ( $1793 \pm 1507$  pg/mmol,  $n = 17$ ) when compared to the participants with IgAVN ( $p = 0.0035$ ) and HC ( $p < 0.0001$ ), as seen in Figure 2b.



**Figure 2.** Urinary creatinine normalised aldosterone concentration, separated by disease and renal involvement status. The mean concentration for each patient group is denoted. (a) Urinary aldosterone is significantly elevated in IgAV patients compared to HC. (b) When separated by renal involvement,

aldosterone levels in IgAwoN patients were significantly elevated compared to HC and IgAVNs. There was also a slight but insignificant trend for elevated levels in IgAVN compared to HC. \*\*  $p < 0.01$ , \*\*\*  $p < 0.0001$ .

### 3.5. Correlation to Proteinuria

The correlation between urinary angiotensinogen, aldosterone and the extent of proteinuria is shown in Table 2. Angiotensinogen was moderately positively correlated ( $r = 0.502$ ,  $p = 0.009$ ) and aldosterone had a low negative correlation with proteinuria values ( $r = -0.444$ ,  $p = 0.023$ ).

**Table 2.** Correlation of urinary angiotensinogen and aldosterone to proteinuria.

	Pearson r	p-Value
Urinary AGT/Cr	0.502	0.009
Urinary aldosterone/Cr	−0.444	0.023

## 4. Discussion

In this study, a cohort of 40 paediatric IgAV patients had constituents of the RAAS pathway, renin, angiotensinogen and aldosterone, which was quantified in urine samples. We evaluated their mechanistic role in IgAV associated nephritis. This study found no differences in the urinary renin concentration or plasma; however, urinary angiotensinogen was significantly elevated in patients with IgAV nephritis. Interestingly, aldosterone was significantly elevated in patients with IgAV without nephritis, and this warrants further investigation. This study offers a characterisation of these urinary components of RAAS in a cross-sectional paediatric IgAV cohort, and it offers preliminary insight into guiding therapeutic RAAS inhibition in this disease.

The relationship between urinary angiotensinogen concentrations and nephritis is well established, and it is a well-recognised marker of intrarenal RAAS activity [6,18–20]. Alongside hepatic production of AGT, the RAAS pathway is also entrenched within the renal system, with angiotensinogen produced locally via the proximal tubule cells, renin being primarily produced in juxtaglomerular cells and ACE being abundant within the kidney [18,21,22]. In this study, urinary AGT was found at a 4.3-fold greater concentration in IgAVN compared to HC and a 2.9-fold greater concentration in IgAwoN compared to HC. This indicates that intrarenal RAAS is activated even where renal inflammation is not evident, and the effect becomes more pronounced once nephritis has evolved. These findings imply that many children have activation of RAAS even without overt nephritis, and that elevated urinary AGT may have a key role in the disease [7,18,21,23,24]. It could be that the vascular inflammation activates the RAAS system triggering AGT production, which would potentially explain the lack of renin dysregulation. Previous studies have also observed elevated urinary AGT levels in paediatric IgAVN [10] and IgAV [25], with a proteomic study investigating serum levels of AGT showing that circulating concentrations of AGT were increased in IgAVN [26]. In glomerular histology samples in IgAN, the AGT concentration was strongly correlated with glomerular angiotensin II, TGF- $\beta$  (a trigger of renal fibrosis) and proteinuria. It has also been demonstrated in patients with IgAN that the M235T polymorphism in AGT exhibits elevated levels of circulating AGT, which is associated with an elevated risk of renal disease progression and adverse cardiac outcomes [27–29]. Downstream proteins in the RAAS pathway, such as ACE, which acts on AGT to produce angiotensin I and II, have been found to contain a polymorphism in their encoding genes, affecting the expression and circulating concentrations of these proteins [27]. Previous work has demonstrated that the use of ACEi in IgAN results in a drop in AGT concentration and improved renal outcomes [30]. This suggests that early ACEi may be a possible intervention in patients with IgAV who are at a high risk of evolving nephritis.

The significant elevation in the urinary concentration of aldosterone of IgAwoN patients is a novel finding and, if these findings are replicable, it suggests that aldosterone

may play a role in the early vasculitic process during the acute phase of IgAV [11]. There is an established body of research demonstrating that aldosterone has a direct effect on the vasculature, with aldosterone receptors being expressed in endothelial cells and vascular smooth muscle cells in addition to renal specific, podocytes and mesangial cells [31,32]. Vascular inflammation induced by aldosterone is a result of oxidative stress and inflammatory cytokines [11,31,32]. When aldosterone is infused for 12 h, inflammatory cytokines IL-6 and IL12 are elevated [32]. Activation of the aldosterone or mineralocorticoid receptor triggers the generation of reactive oxygen species, such as superoxide and hydrogen peroxide, triggering the transcription of proinflammatory transcription factors and increased NADPH oxidase activity, which then complements angiotensinogen mediated activation of NADPH oxidase [31]. All of these factors lead to vascular inflammation and leukocyte adhesion [11].

A potential explanation for the elevated levels of aldosterone and AGT in IgAV may be the elevated renal levels of IgA, which has been demonstrated to increase the expression of aldosterone synthase in mesangial cells. As a result, concentrations of TGF- $\beta$  and AGT become elevated, which may aggravate inflammation [33]. There is also evidence that the galactose-deficient form of IgA (gd-IgA), associated with the early pathogenesis of IgAVN and IgAN, form immune complexes and deposit within the mesangium. This elevated gd-IgA concentration is also associated with elevated oxidative stress and the generation of superoxide, potentially exacerbating the oxidative damage caused by the RAAS pathway or triggering RAAS [34]. This study does have limitations, including the small, heterogenous cohort, and the cross-sectional study design. This may have influenced the findings, and future work would benefit from longitudinal sample collection to determine the precise relationship, as well as the timing of onset and evolution of nephritis, of the RAAS system has in IgAV. Despite these limitations, this study contributes to the literature to demonstrate that there are measurable components of the RAAS system in children with IgAV. This area of study warrants further investigation.

## 5. Conclusions

This study demonstrates elevated urinary angiotensinogen and aldosterone in patients with IgAV, which supports the suggestion that the RAAS system has a key contributory role to the vascular inflammation associated with this disease. These findings offer preliminary evidence to direct further studies to determine whether the early use of RAAS inhibitors may reduce the risk of developing nephritis in children with IgAV.

**Author Contributions:** All authors declare that this is an original manuscript and that they meet the criteria for authorship. Conceptualization: L.O. and A.J.C.; investigation: A.J.C., J.M., D.J.H. and S.J.N.; formal analysis: A.J.C. and J.M.; manuscript preparation: A.J.C., J.M. and L.O.; manuscript review and revisions: A.J.C., J.M., D.J.H., S.J.N. and L.O.; funding acquisition: L.O.; supervision: L.O. All authors have read and agreed to the published version of the manuscript.

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

**Informed Consent Statement:** Informed consent was obtained from all subjects (or their parents/guardians) involved in the study.

**Data Availability Statement:** The datasets analysed in this study are available from the corresponding author on reasonable request.



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**Conflicts of Interest:** The authors declare no conflict of interest.

### Abbreviations

ACE	angiotensin converting enzyme
ACEi	angiotensin converting enzyme inhibitors
AGT	Angiotensinogen
CKD	Chronic Kidney Disease
Cr	Creatinine
ELISA	Enzyme-linked immunosorbent assays
HC	Healthy Control
IgA	Immunoglobulin A
IgAV	Immunoglobulin A Vasculitis
IgAVN	Immunoglobulin A Vasculitis-Nephritis
IgAVoN	Immunoglobulin A Vasculitis without Nephritis
IgAN	Immunoglobulin A Nephropathy
RAAS	Renin-angiotensinogen-aldosterone system
UACR	Urine albumin creatinine ratio

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