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Circulating Immune Biomarkers for Differential Diagnosis of Alport Syndrome and Nephrotic-form Glomerulonephritis in Children

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Abstract

Background

Alport syndrome (AS) may clinically overlap with nephrotic-form chronic glomerulonephritis (CGN), particularly when proteinuria and renal dysfunction become prominent, potentially delaying recognition of hereditary kidney disease. This study evaluated circulating immunological and immunogenetic markers as potential tools for differentiating pediatric AS from CGN and for characterizing renal involvement.

Methods

In this prospective study, 90 children were evaluated, including 30 with genetically confirmed AS, 35 with nephrotic-form CGN, and 25 healthy controls. The AS cohort comprised 14 children with isolated AS and 16 with AS associated with nephrotic syndrome (AS + NS). Serum IL-2, C3, and C4, renal function, proteinuria, and microscopic hematuria were assessed. HLA-DRB1 polymorphisms were analyzed. Associations between immunological markers and estimated glomerular filtration rate (eGFR) were assessed, and receiver operating characteristic (ROC) analysis was performed to evaluate discriminatory performance.

Results

Renal impairment was most frequent in AS + NS (87.5%), followed by isolated AS (64.3%) and CGN (20.0%; $p < 0.001$), with the lowest eGFR in AS + NS (median, 41.4 mL/min/1.73 m²). IL-2, C3, and C4 differed significantly among groups, with IL-2 showing the largest effect size ($\varepsilon^2=0.874$). In the pooled AS cohort, IL-2 was inversely correlated with eGFR ($r = -0.629$, $p < 0.001$), whereas C3 and C4 showed positive correlations. ROC analysis demonstrated excellent discrimination of AS from CGN by IL-2 (AUC = 1.000) and C3 (AUC = 0.970). IL-2 also demonstrated AUC = 1.000 for isolated AS versus CGN and AS + NS versus CGN, while C3 showed AUCs of 1.000 and 0.943, respectively. C4 showed weaker discriminatory performance and was not significant for pooled AS versus CGN (AUC = 0.588, $p = 0.226$). HLA-DRB1*04 was more frequent in AS than in controls (OR = 4.89, 95% CI 1.19–20.03), but the association was not significant after Bonferroni correction.

Conclusion

Pediatric AS demonstrated distinct immunological profiles compared with nephrotic-form CGN, particularly in the AS + NS phenotype. IL-2 and C3 showed excellent discriminatory performance and may represent promising candidate biomarkers for differentiating AS from CGN in children with overlapping clinical presentations. Their association with renal function further suggests potential value

in characterizing disease severity. These findings require validation in larger, independent cohorts before clinical implementation.

Key Message

IL-2 and C3 demonstrated excellent discriminatory performance for differentiating pediatric Alport syndrome from nephrotic-form chronic glomerulonephritis, with IL-2 showing the strongest association with renal dysfunction. The most pronounced clinical and immunological abnormalities were observed in the AS + NS phenotype. These findings identify IL-2 and C3 as promising candidate biomarkers for differential diagnosis and characterization of renal disease severity in pediatric Alport syndrome, while the HLA-DRB1*04 association remains exploratory.

Introduction

Alport syndrome (AS) is a progressive hereditary disorder of the glomerular basement membrane (GBM) caused by pathogenic variants in *COL4A3, COL4A4*, or *COL4A5*, which encode the α3, α4, and α5 chains of type IV collagen. Disruption of the collagen IV α3α4α5 network compromises GBM integrity and contributes to podocyte injury, progressive glomerulosclerosis, tubulointerstitial fibrosis, and nephron loss. Clinically, AS ranges from persistent hematuria to progressive proteinuria, declining glomerular filtration rate (GFR), chronic kidney disease (CKD), and kidney failure; sensorineural hearing loss and ocular abnormalities may also occur. The clinical course is heterogeneous because of differences in inheritance, genotype, variant characteristics, and genotype–phenotype interactions [1–4]. Advances in molecular diagnostics have substantially improved the recognition of AS and other hereditary kidney diseases. Current international recommendations emphasize genetic testing in patients with suspected AS, together with clinical and family-history assessment and longitudinal monitoring of renal function. Early molecular diagnosis is particularly important in children because it can establish the diagnosis before irreversible renal damage develops, facilitate family evaluation, and support timely renoprotective management [1,5–7]. Nevertheless, the phenotypic spectrum of *COL4A3–COL4A5*-related disease may overlap with other glomerular disorders, particularly when proteinuria becomes prominent or a nephrotic phenotype develops, potentially complicating recognition of the underlying hereditary disorder [3,4,8]. Although the primary defect in AS is structural, accumulating evidence indicates that progressive renal injury involves secondary inflammatory and profibrotic mechanisms. Abnormal GBM biomechanics and cellular stress can activate inflammatory signaling, macrophage recruitment, extracellular-matrix remodeling, and fibrotic pathways. Experimental studies have implicated transforming growth factor-β, NF-κB, IL-11, and cGAS-STING signaling in the progression of Alport nephropathy, while increased renal macrophage density has been associated with renal dysfunction and progression toward kidney failure [9–13]. Complement activation may represent an additional mechanism linking glomerular injury with inflammation and fibrosis. The complement system can contribute to endothelial and podocyte injury, leukocyte recruitment, cytokine amplification, and tissue remodeling [14–16]. However, the clinical significance of circulating C3 and C4 concentrations in children with AS remains insufficiently characterized. Potential biomarkers of renal injury have received increasing attention in AS. A review of

candidate renal biomarkers highlighted the need for markers that can better characterize renal injury and disease progression in affected patients [17]. More broadly, early identification of albuminuria is clinically relevant because it can facilitate risk assessment and earlier management of kidney disease. A recent American Journal of Kidney Diseases *study* demonstrated that improving access to urinary albumin-creatinine ratio testing substantially increased albuminuria screening and facilitated subsequent clinical follow-up and kidney-protective management in adults at increased risk for CKD [18]. Although these findings were not specific to AS or pediatric populations, they reinforce the importance of identifying renal involvement before substantial loss of kidney function occurs. Adaptive immune mechanisms may provide an additional explanation for the clinical heterogeneity of AS. Interleukin-2 (IL-2) is a central regulator of T-cell activation, proliferation, differentiation, survival, and regulatory T-cell homeostasis. Altered IL-2 signaling has been implicated in chronic inflammatory and autoimmune conditions, but its relationship with renal dysfunction in children with genetically confirmed AS remains poorly defined [19–22]. Similarly, host immunogenetic factors may modify immune responses and contribute to interindividual differences in disease expression. The human leukocyte antigen (HLA) system has a central role in antigen presentation and adaptive immune regulation. In an early study of unrelated patients with AS, Donadi et al. reported a significantly higher frequency of HLA-DR2 than in population controls, suggesting a possible association between HLA variation and AS [23]. However, this finding involved HLA-DR2 rather than specific HLA-DRB1 alleles associated with renal disease severity, and the contribution of individual HLA-DRB1 polymorphisms to pediatric AS remains poorly characterized. These observations indicate an important knowledge gap concerning the interaction between immune abnormalities, complement alterations, and the renal phenotype of pediatric AS, particularly in children with a nephrotic presentation. Importantly, children with isolated AS and those with AS-associated nephrotic syndrome (AS + NS) may represent clinically distinct phenotypes, with the latter potentially reflecting more advanced renal involvement. Comparative assessment of these two AS phenotypes against children with chronic glomerulonephritis with nephrotic syndrome (CGN-NS) may help determine whether circulating immune abnormalities are preferentially associated with the hereditary Alport phenotype rather than with proteinuria or nephrotic syndrome alone.

Therefore, the present study aimed to characterize clinical, renal, immunological, and immunogenetic differences among children with isolated AS, AS + NS, CGN-NS, and healthy controls. We specifically investigated serum IL-2, C3, and C4 in relation to renal function and evaluated their discriminatory performance using receiver operating characteristic (ROC) analysis. We further examined HLA-DRB1 polymorphisms and their potential association with AS. This integrated approach was designed to identify candidate circulating biomarkers that may complement conventional clinical and molecular parameters in the differential characterization of pediatric AS.

Materials and Methods

This observational cross-sectional study was conducted at the Department of Hospital Pediatrics, Andijan State Medical Institute, in collaboration with the Andijan Regional Children's Multidisciplinary Medical Center, Uzbekistan. Between September 2023 and June 2025, children with suspected

hereditary nephropathy were consecutively screened. Alport syndrome (AS) was diagnosed according to contemporary international recommendations and confirmed by molecular genetic testing. Thirty children with genetically confirmed AS were enrolled and classified into isolated AS and AS associated with nephrotic syndrome (AS + NS). A control group comprised 25 age- and sex-matched apparently healthy children. An additional comparison group included 35 children with nephrotic-form chronic glomerulonephritis (CGN).Inclusion criteria were age < 18 years, genetically confirmed AS, complete clinical and laboratory data, and availability of serum samples for immunological analysis. Exclusion criteria included secondary glomerular diseases, congenital kidney or urinary tract anomalies unrelated to AS, previous kidney transplantation, active infection, malignancy, severe systemic autoimmune disease, and incomplete medical records.

Diagnosis of Alport Syndrome and Molecular Genetic Analysis

Diagnosis was based on clinical assessment, including medical and family history, pedigree analysis, physical examination, urinalysis, renal function assessment, renal ultrasonography, pure-tone audiometry, and ophthalmological examination. Genomic DNA was extracted from peripheral venous blood, and whole-exome sequencing (WES) was performed at SmartGen Molecular Genetics Laboratory (Uzbekistan). Sequencing data were aligned to the GRCh38 (hg38) reference genome, followed by variant calling, annotation, and quality filtering.Variants were interpreted according to American College of Medical Genetics and Genomics (ACMG) criteria. Only patients carrying pathogenic or likely pathogenic variants in COL4A3, COL4A4, or COL4A5 were included. Pathogenic variants were identified in COL4A3 in 17 (56.7%), COL4A5 in 12 (40.0%), and COL4A4 in 1 (3.3%) patient.

Clinical and Laboratory Assessment

Clinical assessment included demographic characteristics, age at disease onset, family history, physical examination, and renal and extrarenal manifestations. CGN was diagnosed based on persistent nephrotic-range proteinuria, hypoproteinemia, dysproteinemia, hypercholesterolemia, and edema, with secondary glomerular disorders excluded clinically and laboratory-wise. Kidney biopsy was not performed; therefore, the histological subtype of glomerulonephritis was not determined. Renal function was assessed using serum creatinine and estimated glomerular filtration rate (eGFR), calculated with the updated bedside Schwartz Eq. (2009): eGFR = 0.413 × height (cm) / serum creatinine (mg/dL). Chronic kidney disease stages were classified according to KDIGO guidelines. Because standing height was not measured in healthy controls, eGFR was not calculated for this group, and renal function was characterized by serum creatinine. Patients with AS were classified as having isolated AS or AS + NS. Nephrotic syndrome was defined according to internationally accepted pediatric criteria, including nephrotic-range proteinuria, hypoalbuminemia, hyperlipidemia, and generalized edema.

Immunological Assessment

Peripheral venous blood samples were collected under standardized conditions before immunosuppressive therapy or during routine clinical assessment. Serum was separated by centrifugation and analyzed immediately or stored at − 80°C. Serum C3 and C4 concentrations were measured by immunoturbidimetry using an automated biochemical analyzer. Serum IL-2 concentrations were determined using a commercial sandwich ELISA kit (Protein Contour LLC and Cytokine Ltd., St. Petersburg, Russian Federation) according to the manufacturer's protocol. IL-2 concentrations were expressed as pg/mL. All immunological analyses were performed in certified laboratories using standardized procedures and internal quality-control measures.

Immunogenetic Analysis

HLA class II genotyping, with particular emphasis on HLA-DRB1, was performed using polymerase chain reaction with sequence-specific primers (PCR-SSP). Genomic DNA was extracted from peripheral blood leukocytes, and amplified fragments were analyzed according to the manufacturer's recommendations. The frequencies of HLA-DRB1*04 and HLA-DRB1*07 were compared between children with AS and healthy controls to assess their potential associations with disease susceptibility and clinical phenotype.

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics version 27.0. Normality and variance homogeneity were assessed using the Shapiro–Wilk and Levene tests. Because most variables were non-normally distributed, non-parametric methods were used. Continuous variables are presented as median [Q1; Q3], with mean ± SD additionally reported. Between-group comparisons were performed using the Kruskal–Wallis test followed by pairwise Mann–Whitney U tests with Bonferroni correction. Categorical variables were analyzed using Pearson's chi-square, Fisher's exact, or Fisher–Freeman–Halton tests, as appropriate. Effect sizes were reported as ε^2, Rosenthal's *r*, and Cramér's V. Associations were assessed using Spearman's rank correlations, with partial correlations adjusted for age. ROC analysis was performed to evaluate IL-2, C3, and C4 for discrimination between isolated AS, AS + NS, pooled AS, and CGN. AUC, sensitivity, specificity, and optimal cut-off values based on the Youden index were calculated. Odds ratios with 95% confidence intervals were calculated using the Woolf method. Complete-case analysis was performed without imputation. A two-sided $p < 0.05$ was considered statistically significant.

Results

Clinical and Renal Characteristics of the Study Population

The study included 90 children: 35 with chronic glomerulonephritis (CGN), 30 with Alport syndrome (AS), including 14 with isolated AS and 16 with AS associated with nephrotic syndrome (AS + NS), and 25 healthy controls. The study groups were comparable with respect to age ($H = 1.211$, $p = 0.750$). However, sex distribution differed significantly among the groups ($\chi^2=8.084$, $p = 0.044$), with a predominance of

males in the AS + NS group (87.5%). A marked difference in family history of renal failure was observed among the clinical groups ($p < 0.001$). A positive family history was reported in all children with isolated AS (100%) and in 81.8% of children with AS + NS, compared with 10.7% of CGN patients for whom family-history data were available. The effect size for this association was large (Cramér's V = 0.844). Significant between-group differences were also observed in the prevalence of proteinuria, leukocyturia, edema, arterial hypertension, and impaired renal function (all $p < 0.001$). Impaired renal function was particularly frequent in children with AS + NS, occurring in 87.5% of patients, compared with 64.3% of those with isolated AS and 20.0% of those with CGN. Similarly, arterial hypertension was most frequent in the AS + NS group (87.5%), followed by isolated AS (64.3%) and CGN (31.4%). Detailed demographic and clinical characteristics are presented in Table 1.

Table 1
Demographic and clinical characteristics of the study groups

Variable	**Healthy controls (n = 25)**	**CGN (n = 35)**	**AS (n = 14)**	**AS + NS (n = 16)**	**P**	**Effect size**
Male sex, n (%)	12 (48.0)	24 (68.6)	11 (78.6)	14 (87.5)	0.044	V = 0.300
Age, years, Me (Q1; Q3)	11.0 (6.0; 15.0)	10.0 (7.5; 12.0)	9.5 (6.3; 16.8)	12.0 (6.0; 14.3)	0.750	$\varepsilon^2<0.01$
Age, years, M ± SD	10.80 ± 4.80	9.80 ± 3.17	11.14 ± 5.49	11.00 ± 4.52	—	—
Height, cm, Me (Q1; Q3)	NM	132 (122; 142)	123 (119; 149)	140 (124; 158)	0.588	$\varepsilon^2<0.01$
Renal failure in relatives[a], n (%)	0/25 (0.0)	3/28 (10.7)	9/9 (100.0)	9/11 (81.8)	< 0.001	V = 0.844
Proteinuria, n (%)	0 (0.0)	34 (97.1)	13 (92.9)	14 (87.5)	< 0.001	V = 0.902
Leukocyturia, n (%)	0 (0.0)	13 (37.1)	9 (64.3)	13 (81.3)	< 0.001	V = 0.595
Edema, n (%)	0 (0.0)	20 (57.1)	4 (28.6)	11 (68.8)	< 0.001	V = 0.552
Arterial hypertension[b], n (%)	0 (0.0)	11 (31.4)	9 (64.3)	14 (87.5)	< 0.001	V = 0.639
Impaired renal function, n (%)	0 (0.0)	7 (20.0)	9 (64.3)	14 (87.5)	< 0.001	V = 0.687

Note: Me, median; Q1–Q3, interquartile range; M ± SD, mean ± standard deviation; NM, not measured. [a] Family-history data were unavailable for 17 participants. [b] Arterial hypertension was defined as blood pressure ≥ 95th percentile for sex, age, and height.

Renal Function and Proteinuria

Serum creatinine concentrations differed significantly among the study groups (H = 45.057, p < 0.001, ε²=0.489), indicating a large between-group effect. The median creatinine concentration was 44.0 (33.0–62.0) μmol/L in healthy controls, 70.0 (64.5–77.0) μmol/L in children with CGN, 78.0 (56.0–110.0) μmol/L in those with isolated AS, and 128.5 (75.8–151.5) μmol/L in the AS + NS group. The highest creatinine concentrations were therefore observed among children with AS + NS, consistent with more pronounced renal impairment in this phenotype. Proteinuria also differed significantly across the study groups (H = 57.601, p < 0.001, ε²=0.635). The highest median proteinuria was observed in the CGN group, at 3.30 (3.30–6.60) g/L, compared with 0.33 (0.12–0.91) g/L in isolated AS and 0.99 (0.16–3.30) g/L in AS + NS. No statistically significant difference was observed between the isolated AS and AS + NS groups (p = 1.000). The corresponding findings are summarized in (Table 2).

Table 2
Serum creatinine and proteinuria in the study groups

Variable	Healthy controls (n = 25)	CGN (n = 35)	AS (n = 14)	AS + NS (n = 16)	H	p	ε²
Creatinine, μmol/L, Me (Q1; Q3)	44.0 (33.0; 62.0)	70.0 (64.5; 77.0)	78.0 (56.0; 110.0)	128.5 (75.8; 151.5)	45.057	< 0.001	0.489
Creatinine, M ± SD	46.4 ± 13.9	74.6 ± 19.5	83.1 ± 33.9	127.6 ± 57.2	—	—	—
Proteinuria, g/L, Me (Q1; Q3)	0.03 (0.03; 0.03)	3.30 (3.30; 6.60)	0.33 (0.12; 0.91)	0.99 (0.16; 3.30)	57.601	< 0.001	0.635
Proteinuria, M ± SD	0.03 ± 0.00	4.75 ± 3.37	1.16 ± 2.56	2.29 ± 2.77	—	—	—

Estimated Glomerular Filtration Rate and CKD Stages

Estimated glomerular filtration rate (eGFR) differed significantly among the clinical groups (H = 15.684, p < 0.001, ε²=0.221). The median eGFR was 69.1 (57.1–73.9) mL/min/1.73 m² in children with CGN, 62.6 (49.6–78.6) mL/min/1.73 m² in those with isolated AS, and 41.4 (33.3–58.4) mL/min/1.73 m² in children with AS + NS. Thus, the lowest renal function was observed in the AS + NS group. The distribution of CKD stages differed significantly among the clinical groups (χ²=25.012, p = 0.002, Cramér's V = 0.439). More advanced CKD stages were particularly frequent among children with AS + NS. CKD C3b was observed in 43.8% of children with AS + NS, compared with 5.7% of those with CGN and 7.1% of those with isolated AS. CKD C4 was present in 12.5% of the AS + NS group and 7.1% of the isolated AS group, but was not observed among children with CGN. An eGFR below 60 mL/min/1.73 m² was observed in 31.4% of children with CGN, 42.9% of those with isolated AS, and 81.2% of those with AS + NS (χ²=11.045, p =

0.004, Cramér's V = 0.412). These findings indicate a substantially greater burden of renal dysfunction in children with AS + NS compared with the other clinical groups (Table 3).

Table 3
eGFR and CKD stages in the clinical groups

Variable	CGN (n = 35)	AS (n = 14)	AS + NS (n = 16)	P	Effect size
eGFR, Me [Q1; Q3]	69.1 [57.1; 73.9]	62.6 [49.6; 78.6]	41.4 [33.3; 58.4]	< 0.001	ε^2=0.221
eGFR, M ± SD	67.7 ± 14.5	68.8 ± 30.7	46.1 ± 17.5	—	—
CKD C1, n (%)	2 (5.7)	3 (21.4)	0 (0.0)	0.002	V = 0.439
CKD C2, n (%)	22 (62.9)	5 (35.7)	3 (18.8)	—	—
CKD C3a, n (%)	9 (25.7)	4 (28.6)	4 (25.0)	—	—
CKD C3b, n (%)	2 (5.7)	1 (7.1)	7 (43.8)	—	—
CKD C4, n (%)	0 (0.0)	1 (7.1)	2 (12.5)	—	—
eGFR < 60, n (%)	11 (31.4)	6 (42.9)	13 (81.2)	0.004	V = 0.412

Immunological Profile

Significant differences among the study groups were identified for serum C3, C4, and IL-2 concentrations. IL-2 demonstrated the largest effect size (H = 78.141, p < 0.001, ε^2=0.874). Median IL-2 concentrations were 2.80 (2.70–2.86) pg/mL in healthy controls, 2.23 (2.21–2.31) pg/mL in children with CGN, 18.28 (18.02–18.77) pg/mL in isolated AS, and 25.21 (22.42–28.33) pg/mL in AS + NS. IL-2 concentrations were significantly higher in both AS phenotypes than in CGN and healthy controls, with the highest concentrations observed in AS + NS. C3 concentrations also differed significantly among the groups (H = 76.017, p < 0.001, ε^2=0.849). The median C3 concentration was 1.80 (1.80–1.90) g/L in healthy controls, 0.92 (0.78–0.97) g/L in CGN, 1.64 (1.62–1.68) g/L in isolated AS, and 1.53 (1.38–1.57) g/L in AS + NS. C3 concentrations were significantly lower in CGN than in the AS groups, while remaining below the values observed in healthy controls. C4 concentrations likewise differed significantly among the groups (H = 71.074, p < 0.001, ε^2=0.792). Median C4 concentrations were 0.40 (0.37–0.41) g/L in healthy controls, 0.21 (0.18–0.23) g/L in CGN, 0.29 (0.27–0.31) g/L in isolated AS, and 0.19 (0.17–0.20) g/L in AS + NS. Pairwise comparisons demonstrated significant differences between the clinical groups, although the direction and magnitude of these differences varied according to AS phenotype (Table 4).

Table 4
Immunological parameters in the study groups

Variable	Healthy controls	CGN	AS	AS + NS	H	p	ε²
C3, g/L, Me (Q1; Q3)	1.80 (1.80; 1.90)	0.92 (0.78; 0.97)▯	1.64 (1.62; 1.68)▯▯	1.53 (1.38; 1.57)▯▯▯	76.017	< 0.001	0.849
C4, g/L, Me (Q1; Q3)	0.40 (0.37; 0.41)	0.21 (0.18; 0.23)▯	0.29 (0.27; 0.31)▯▯	0.19 (0.17; 0.20)▯▯	71.074	< 0.001	0.792
IL-2, pg/mL, Me (Q1; Q3)	2.80 (2.70; 2.86)	2.23 (2.21; 2.31)▯	18.28 (18.02; 18.77)▯▯	25.21 (22.42; 28.33)▯▯▯	78.141	< 0.001	0.874

▯ *p < 0.001 versus healthy controls;* ▯ *p < 0.001 versus CGN;* ▯ *p < 0.001 versus isolated AS.*

Immunogenetic Findings

*25 healthy controls. HLA-DRB1*04 was detected in 12 children with AS (40.0%) compared with 3 healthy controls (12.0%). The unadjusted analysis demonstrated a significant association between HLA-DRB1*04 carriage and AS (OR = 4.89, 95% CI: 1.19–20.03; p = 0.032). However, this association did not remain statistically significant after Bonferroni correction (adjusted p = 0.065).*

*HLA-DRB1*07 was detected in 10 children with AS (33.3%) and 4 healthy controls (16.0%). The difference was not statistically significant (OR = 2.62, 95% CI: 0.71–9.74; p = 0.215), and remained non-significant after adjustment for multiple comparisons (adjusted p = 0.430). Thus, the present data provide only preliminary evidence for an association between HLA-DRB1*04* and AS, which requires confirmation in a larger cohort (Table 5).

Table 5
Carrier frequencies of HLA-DRB1 alleles

Allele	AS (n = 30), n (%)	Healthy controls (n = 25), n (%)	OR	95% CI	p	Adjusted p
HLA-DRB1*04	12 (40.0)	3 (12.0)	4.89	1.19–20.03	0.032	0.065
HLA-DRB1*07	10 (33.3)	4 (16.0)	2.62	0.71–9.74	0.215	0.430

Diagnostic Performance of Immunological biomarkers

Receiver operating characteristic (ROC) analysis was performed to assess the discriminatory performance of IL-2, C3, and C4 concentrations for differentiating children with Alport syndrome from those with chronic glomerulonephritis. The analysis was conducted separately for isolated AS, AS associated with nephrotic syndrome (AS + NS), and the pooled AS cohort.For isolated AS versus CGN, IL-2 and C3 demonstrated perfect discriminatory performance, with an area under the curve (AUC) of 1.000

for both biomarkers ($p < 0.001$). An IL-2 concentration > 2.80 pg/mL yielded 100.0% sensitivity and 100.0% specificity (Youden's J = 1.000). Similarly, a C3 concentration > 1.24 g/L provided 100.0% sensitivity and 100.0% specificity (Youden's J = 1.000). C4 also demonstrated excellent discrimination, with an AUC of 0.993 ($p < 0.001$); a cut-off value > 0.24 g/L yielded 100.0% sensitivity and 94.3% specificity (Youden's J = 0.943) (Table 5). In the AS + NS versus CGN comparison, IL-2 again showed perfect discriminatory ability (AUC = 1.000, $p < 0.001$), with 100.0% sensitivity and specificity at a cut-off > 2.80 pg/mL (Youden's J = 1.000). C3 demonstrated excellent discrimination (AUC = 0.943, $p < 0.001$), with a cut-off > 1.146 g/L providing 100.0% sensitivity and 94.3% specificity (Youden's J = 0.943). In contrast, C4 showed only moderate discriminatory performance (AUC = 0.773, $p = 0.002$), with a cut-off ≤ 0.198 g/L yielding 75.0% sensitivity and 54.3% specificity (Youden's J = 0.293). When all AS cases, including both isolated AS and AS + NS, were analyzed as a pooled cohort, IL-2 maintained perfect discrimination from CGN (AUC = 1.000, $p < 0.001$), with 100.0% sensitivity and specificity at a cut-off > 2.80 pg/mL (Youden's J = 1.000). C3 also demonstrated excellent

discriminatory performance (AUC = 0.970, $p < 0.001$), with 96.7% sensitivity and 100.0% specificity at a cut-off > 1.24 g/L (Youden's J = 0.967). However, C4 did not demonstrate statistically significant discrimination in the pooled AS cohort (AUC = 0.588, $p = 0.226$), and no optimal cut-off was identified (Table 6).

Table 6
Receiver operating characteristic (ROC) analysis of immunological biomarkers for discrimination between Alport syndrome and chronic glomerulonephritis

Biomarker	Comparison	AUC	p-value	Optimal cut-off	Sensitivity, %	Specificity, %	Youden's J
IL-2	AS vs CGN	1.000	< 0.001	> 2.80 pg/mL	100.0	100.0	1.000
C3	AS vs CGN	1.000	< 0.001	> 1.24 g/L	100.0	100.0	1.000
C4	AS vs CGN	0.993	< 0.001	> 0.24 g/L	100.0	94.3	0.943
IL-2	AS + NS vs CGN	1.000	< 0.001	> 2.80 pg/mL	100.0	100.0	1.000
C3	AS + NS vs CGN	0.943	< 0.001	> 1.146 g/L	100.0	94.3	0.943
C4	AS + NS vs CGN	0.773	0.002	≤ 0.198 g/L	75.0	54.3	0.293
IL-2	Pooled AS vs CGN	1.000	< 0.001	> 2.80 pg/mL	100.0	100.0	1.000
C3	Pooled AS vs CGN	0.970	< 0.001	> 1.24 g/L	96.7	100.0	0.967
C4	Pooled AS vs CGN	0.588	0.226	—	—	—	—

Abbreviations: AUC, area under the ROC curve; AS, Alport syndrome; AS + NS, Alport syndrome associated with nephrotic syndrome; CGN, chronic glomerulonephritis.

The ROC analysis demonstrated excellent discriminatory performance of IL-2 and C3 for differentiating AS phenotypes from CGN. IL-2 showed perfect discrimination in isolated AS, AS + NS, and the pooled AS cohort, with an AUC of 1.000 in all three comparisons. C3 also demonstrated excellent discriminatory performance, with AUC values of 1.000, 0.943, and 0.970 for isolated AS, AS + NS, and pooled AS, respectively. In contrast, the discriminatory performance of C4 varied across the clinical phenotypes and was not statistically significant in the pooled AS cohort (AUC = 0.588, p = 0.226). These findings are illustrated in(Fig. 1).

Overall, IL-2 demonstrated the most consistent diagnostic performance across all AS phenotypes, achieving an AUC of 1.000 for isolated AS, AS + NS, and the pooled AS cohort. C3 also showed excellent discriminatory ability, particularly in the pooled AS cohort, whereas the diagnostic performance of C4 was less consistent and was not statistically significant when the entire AS cohort was analyzed. These findings indicate that IL-2 and C3 may have greater potential as biomarkers for differentiating AS, including the AS + NS phenotype, from CGN.

Correlations Between Immunological Markers and Renal Function

Within the pooled AS cohort, IL-2 demonstrated a significant negative correlation with eGFR (r = − 0.629, 95% CI − 0.81 to − 0.35, p < 0.001). In contrast, C3 and C4 were positively correlated with eGFR (r = + 0.422, p = 0.020 and r = + 0.409, p = 0.025, respectively).IL-2 was inversely correlated with both C3 (r = − 0.677, p < 0.001) and C4 (r = − 0.781, p < 0.001), whereas C3 and C4 showed a strong positive correlation (r = + 0.777, p < 0.001).After adjustment for age using partial rank correlation analysis, the associations remained statistically significant for IL-2 and eGFR (r = − 0.642, p < 0.001), C3 and eGFR (r = + 0.477, p = 0.009), and C4 and eGFR (r = + 0.418, p = 0.024).In the isolated AS subgroup, IL-2 demonstrated a strong inverse correlation with eGFR (r = − 0.788, 95% CI − 0.93 to − 0.44, p = 0.001; n = 14) (Fig. 2).

Summary of the Main Findings

Overall, the study demonstrated distinct clinical, renal, immunological, and immunogenetic characteristics across the investigated groups. The AS + NS phenotype was associated with the most pronounced impairment of renal function, as reflected by higher serum creatinine concentrations, lower eGFR, a greater proportion of children with eGFR < 60 mL/min/1.73 m², and a higher prevalence of advanced CKD stages. Among the immunological parameters investigated, IL-2 showed the strongest between-group effect and the most consistent discriminatory performance. IL-2 concentrations were markedly elevated in both isolated AS and AS + NS compared with CGN, and IL-2 demonstrated an AUC of 1.000 in all three ROC analyses. C3 also showed excellent discriminatory performance, particularly in the pooled AS cohort, whereas C4 demonstrated less consistent discrimination. Furthermore, increased IL-2 concentrations and lower C3 and C4 concentrations were significantly associated with reduced eGFR within the AS cohort. Although HLA-DRB1*04 showed an association with AS in the unadjusted analysis, this association did not retain statistical significance after correction for multiple comparisons. Taken together, these findings suggest that IL-2 and C3 represent promising candidate circulating biomarkers for differentiating pediatric AS, including the AS + NS phenotype, from CGN. However, the observed diagnostic performance should be interpreted as preliminary and requires validation in larger, independent pediatric cohorts before clinical application.

Discussion

The differential diagnosis of hereditary and acquired glomerular disease in children remains clinically challenging, particularly when Alport syndrome (AS) presents with substantial proteinuria, nephrotic-range manifestations, or impaired renal function. The present study investigated whether circulating immune parameters could contribute to the distinction between AS phenotypes and chronic glomerulonephritis (CGN). The principal finding was that circulating IL-2 and C3 demonstrated consistently high discriminatory performance for distinguishing AS from CGN, including children with AS associated with nephrotic syndrome (AS + NS). IL-2 showed an area under the receiver operating

characteristic curve (AUC) of 1.000 for isolated AS versus CGN, AS + NS versus CGN, and the pooled AS cohort versus CGN, whereas C3 showed AUC values of 1.000, 0.943, and 0.970, respectively. In contrast, C4 demonstrated less consistent discriminatory performance. These findings suggest that circulating immune parameters, particularly IL-2 and C3, may provide complementary phenotypic information in children in whom the clinical presentation of hereditary and acquired glomerular disease overlaps. AS is fundamentally a hereditary disorder caused by pathogenic variants in *COL4A3, COL4A4*, or *COL4A5*, resulting in abnormalities of the glomerular basement membrane (GBM) [1–8]. The clinical phenotype is heterogeneous, ranging from persistent microscopic hematuria to progressive proteinuria, chronic kidney disease (CKD), and kidney failure [1–8]. This phenotypic variability creates a particular diagnostic challenge when children with AS develop substantial proteinuria or a nephrotic phenotype, because these findings may initially suggest an acquired glomerular disorder. In the present study, the AS + NS group exhibited the most pronounced renal impairment, characterized by higher serum creatinine, lower eGFR, a greater frequency of eGFR < 60 mL/min/1.73 m², and more advanced CKD stages. These findings support the concept that AS associated with nephrotic manifestations represents a clinically severe phenotype within the COL4-related disease spectrum rather than simply an isolated increase in urinary protein excretion [1–8]. The diagnostic findings of the present study are particularly relevant in this context. IL-2 demonstrated complete separation between the AS and CGN distributions in the present cohort, resulting in an AUC of 1.000 for isolated AS and AS + NS. The same threshold (> 2.80 pg/mL) provided 100% sensitivity and 100% specificity in both comparisons. Importantly, the high IL-2 concentrations observed in AS were not simply a reflection of the degree of proteinuria, because median proteinuria was highest in the CGN group, whereas IL-2 concentrations were substantially higher in both AS subgroups. This dissociation suggests that IL-2 may capture biological differences between COL4-related and acquired glomerular disease that are not adequately reflected by proteinuria alone. The performance of C3 further supports the potential value of circulating immune parameters. C3 demonstrated perfect discrimination between isolated AS and CGN (AUC = 1.000) and excellent discrimination between AS + NS and CGN (AUC = 0.943). When the AS cohort was analyzed as a whole, the AUC remained high at 0.970. The consistency of C3 across different AS phenotypes is important because children with AS may present with substantially different degrees of proteinuria and renal dysfunction. Thus, C3 may provide complementary information when clinical features alone do not clearly distinguish hereditary GBM disease from acquired glomerular disease.In contrast, C4 demonstrated a more heterogeneous diagnostic pattern. Although C4 showed excellent discrimination between isolated AS and CGN (AUC = 0.993), its performance declined in AS + NS (AUC = 0.773) and was not statistically significant in the pooled AS cohort (AUC = 0.588, p = 0.226). This inconsistency indicates that C4 is unlikely to function as a reliable stand-alone discriminator across the full clinical spectrum of AS. Nevertheless, the significant association between C4 and eGFR suggests that C4 may still reflect aspects of renal involvement and may have value as part of a multidimensional biomarker profile rather than as an individual diagnostic marker. The marked increase in IL-2 in AS, particularly in AS + NS, provides a potentially important biological observation. IL-2 is a central regulator of T-cell proliferation, differentiation, survival, and immune homeostasis [19–22]. In the present study, IL-2 concentrations were strongly inversely correlated with eGFR in the pooled AS cohort, and this association remained

significant after adjustment for age. A similarly strong inverse association was observed in children with isolated AS. These findings indicate that higher circulating IL-2 concentrations were associated with greater renal functional impairment. However, the cross-sectional design does not establish whether increased IL-2 contributes to renal injury or instead reflects secondary immune activation associated with established kidney dysfunction. Therefore, the present results should be interpreted as evidence of an association rather than causality.The relationship between IL-2 and renal function may be biologically plausible within the broader inflammatory framework of AS. Although AS is initiated by a structural defect in type IV collagen, abnormal GBM composition and progressive glomerular injury may induce secondary cellular stress and inflammatory responses [9–13]. Experimental studies have implicated tubular epithelial responses, macrophage accumulation, and profibrotic pathways in the progression of COL4-related kidney disease [10–13]. In addition, innate immune pathways such as cGAS-STING have been implicated in renal inflammation and injury [32], while chronic inflammatory signaling is increasingly recognized as a component of CKD progression [33]. These mechanisms provide a possible context for the association between circulating IL-2 and renal function observed in the present study. Nevertheless, IL-2 is not specific for AS-related inflammation, and the present study did not directly investigate these intracellular or tissue-level pathways.The complement findings provide additional evidence of altered immune biology in AS. C3 and C4 concentrations differed significantly among the study groups and were positively associated with eGFR. Complement pathways have been implicated in renal inflammation, podocyte injury, endothelial dysfunction, and tissue remodeling [14–18]. However, circulating C3 and C4 concentrations alone cannot establish the presence or extent of intrarenal complement activation. Reduced serum concentrations may reflect consumption, altered synthesis, redistribution, or other systemic processes. Therefore, the present findings should not be interpreted as direct evidence that complement activation is a primary pathogenic mechanism in AS. Future studies incorporating complement activation fragments, urinary complement components, or renal tissue analysis will be required to determine the mechanistic significance of these observations. The inverse correlations between IL-2 and both C3 and C4, together with the strong positive correlation between C3 and C4, indicate coordinated variation among the investigated immune parameters. However, these correlations do not establish a direct mechanistic interaction between IL-2 and complement pathways. Rather, they may reflect the broader biological response associated with progressive renal injury. This distinction is important because AS remains fundamentally a genetic disorder of the GBM [9], and the observed circulating immune abnormalities may represent secondary responses superimposed on the primary COL4-related defect.The AS + NS phenotype deserves particular consideration in relation to differential diagnosis. Children in this subgroup had the highest IL-2 concentrations and the greatest degree of renal impairment. At the same time, proteinuria was not significantly different between isolated AS and AS + NS and was actually highest in the CGN group. Thus, the markedly elevated IL-2 concentrations in AS cannot be explained simply by the amount of urinary protein loss. This finding is particularly relevant to the clinical problem addressed by the present study: a child with AS may develop substantial proteinuria and renal dysfunction that clinically resembles nephrotic-form acquired glomerular disease. The present data suggest that IL-2 and, to a lesser extent, C3 may provide additional biological information in this diagnostic setting. These observations may be particularly valuable when

considering children with suspected hereditary glomerular disease but without an immediately available or definitive molecular diagnosis. Molecular genetic testing remains central to the diagnosis of AS and should not be replaced by circulating immune markers [26]. Nevertheless, genetic testing may not always be immediately accessible, and clinical phenotypes can overlap substantially between hereditary and acquired glomerular disorders. In such circumstances, circulating biomarkers could potentially serve as complementary tools within a broader diagnostic algorithm incorporating family history, urinalysis, proteinuria, renal function, extrarenal manifestations, and molecular genetic testing [1,5,17,26]. The present findings should also be interpreted in the context of the known genetic and phenotypic heterogeneity of AS. Variation in inheritance pattern, genetic background, and genotype may influence disease expression and the rate of renal progression [24, 25]. Autosomal dominant AS, for example, demonstrates considerable clinical and genetic variability [30], while genotype-dependent mechanisms may contribute to differences in disease severity [31]. Such heterogeneity could partly explain why C4 showed less consistent discriminatory performance than IL-2 and C3. It also emphasizes that circulating immune markers are unlikely to replace genetic characterization, but may instead reflect biological consequences of disease that are complementary to genotype. The exploratory HLA analysis provides additional, although preliminary, information. HLA-DRB1*04* was more frequent among children with AS than healthy controls in the unadjusted analysis, with an OR of 4.89. However, this association did not remain statistically significant after Bonferroni correction. HLA polymorphisms may influence antigen presentation and adaptive immune responses, and associations between HLA variation and kidney disease have previously been reported [23,36]. Nevertheless, evidence linking specific HLA alleles to COL4*-related nephropathy remains limited. Accordingly, the HLA-DRB1*04 finding should be regarded as hypothesis-generating rather than confirmatory and requires replication in larger genetically characterized cohorts. The potential clinical relevance of the present findings lies primarily in their application to differential diagnosis rather than treatment selection. Current management of AS emphasizes early recognition, molecular diagnosis, family assessment, and timely renoprotective therapy [27, 34–36]. Emerging approaches directed toward regenerative, anti-inflammatory, and disease-modifying mechanisms further increase the importance of identifying biologically meaningful markers of disease activity [34–36]. However, the present study does not establish IL-2 or C3 as therapeutic targets, nor does it demonstrate their ability to predict treatment response. Their potential role is currently best considered as an adjunctive diagnostic or phenotypic tool. The clinical heterogeneity of AS is further illustrated by reports of unusual presentations and familial associations, including the co-occurrence of AS and diffuse leiomyomatosis [28]. Such observations emphasize the importance of comprehensive clinical assessment and careful evaluation of extrarenal manifestations. In children, combinations of hematuria, proteinuria, impaired renal function, and extrarenal findings may complicate phenotype-based classification [29]. Integration of clinical, genetic, and potentially circulating biomarker data may therefore provide a more comprehensive characterization of individual disease phenotypes and trajectories.

Clinical Implications

From a clinical perspective, the most relevant finding is the consistently high discriminatory performance of IL-2 across the investigated AS phenotypes. The ability of IL-2 to distinguish both isolated AS and AS + NS from CGN suggests that its potential diagnostic value is not restricted to mild or early AS but may also extend to children presenting with a nephrotic phenotype and substantial renal impairment. C3 showed similarly strong performance and may provide complementary information, whereas C4 appears less suitable as an independent discriminator. These findings are particularly relevant because the distinction between hereditary and acquired glomerular disease can be difficult when proteinuria and reduced renal function are prominent. A biomarker profile incorporating IL-2 and C3 could potentially complement conventional clinical assessment and genetic testing. However, the present study does not justify routine clinical use of these markers. The observed thresholds and diagnostic estimates should be regarded as exploratory and require validation in larger, independent pediatric cohorts representing a broader spectrum of glomerular diseases.

Limitations

Several limitations should be considered when interpreting these findings. First, the sample size was relatively small, particularly within the isolated AS and AS + NS subgroups. This limitation may result in imprecise estimates of diagnostic accuracy and may partly explain the apparently perfect AUC values observed for IL-2 and C3. Second, the study was cross-sectional and therefore cannot establish temporal or causal relationships between immune markers and renal dysfunction. Third, the analysis compared AS primarily with CGN and does not establish whether IL-2 or C3 can distinguish AS from the broader spectrum of pediatric nephrotic and glomerular diseases. Fourth, circulating C3 and C4 concentrations do not directly measure intrarenal complement activation. Fifth, although IL-2 demonstrated strong associations with renal function, it is not specific for AS and may be influenced by other inflammatory or systemic processes. Sixth, the study did not determine whether the identified biomarkers provide diagnostic information beyond established variables such as proteinuria, eGFR, family history, and genotype. Finally, the HLA analysis was exploratory and the observed HLA-DRB1*04 association lost statistical significance after correction for multiple testing. Future multicenter prospective studies should therefore evaluate larger pediatric cohorts, including children with other causes of nephrotic syndrome and glomerulonephritis. External validation of the IL-2 and C3 thresholds identified in the present study is particularly important. Longitudinal investigations should also determine whether these markers change with disease progression and whether they provide prognostic information beyond eGFR, proteinuria, and genotype. Studies combining circulating biomarkers with molecular genetic data may ultimately determine whether a multimarker approach improves the diagnostic classification of children with suspected AS.

Conclusion

The present study demonstrates that pediatric Alport syndrome, particularly the AS phenotype associated with nephrotic syndrome, is characterized by distinct renal and circulating immunological features. Children with AS + NS showed the most pronounced renal impairment, while IL-2

concentrations were markedly elevated and C3 and C4 concentrations were significantly altered across the investigated groups. Among the evaluated circulating immune parameters, IL-2 demonstrated the most consistent discriminatory performance, with an AUC of 1.000 for distinguishing isolated AS and AS + NS from CGN. C3 also demonstrated excellent discrimination, with AUC values of 1.000 for isolated AS and 0.943 for AS + NS. In contrast, C4 showed substantially less consistent diagnostic performance, particularly when the entire AS cohort was analyzed. The strong inverse association between IL-2 and eGFR, together with the positive associations of C3 and C4 with renal function, suggests that these circulating immune parameters may reflect biologically relevant differences in renal involvement among children with AS. Importantly, the findings also indicate that IL-2 and C3 may have potential value in the differential characterization of children with COL4-related nephropathy who present with clinical features overlapping with acquired glomerular disease, including nephrotic manifestations. Overall, IL-2 and C3 should be regarded as promising candidate circulating biomarkers rather than established diagnostic tests. Their potential clinical value lies in complementing, rather than replacing, molecular genetic testing and conventional clinical assessment. The high diagnostic estimates observed in this study require validation in larger, independent, prospective cohorts including a broader spectrum of pediatric glomerular diseases. Such validation will be essential to determine whether a combined biomarker approach can improve the early and differential diagnosis of Alport syndrome in children with nephrotic and glomerulonephritic presentations.

Declarations

Ethics approval and consent to participate

This study was conducted in accordance with the principles of the Declaration of Helsinki. Ethical approval was obtained from the Ethics Committee of Andijan State Medical Institute (approval No. 18/80, dated September 7, 2023). The study included 90 children, comprising 35 children with chronic glomerulonephritis (CGN), 30 children with Alport syndrome (AS), and 25 healthy children who served as controls. The AS group comprised 14 children with isolated AS and 16 children with AS accompanied by nephrotic syndrome. Written informed consent was obtained from the parents or legal guardians of all participating children prior to enrollment. All participant data were handled confidentially and used exclusively for research purposes.

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Conflict of Interest

The authors declare that they have no conflicts of interest related to this study.

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Figures

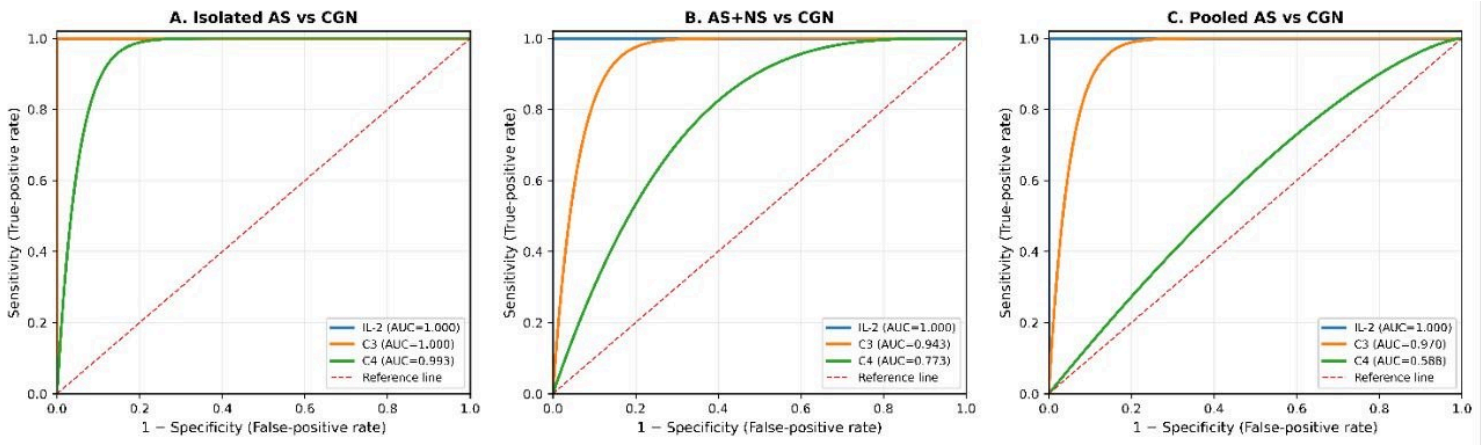


Figure 1

Receiver operating characteristic (ROC) curves of IL-2, C3, and C4 for discrimination between AS phenotypes and CGN.



Figure 2

Spearman correlations between immunological markers and renal function in the AS cohort

Supplementary Files

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