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Optimizing outcomes for children with immune-mediated chronic kidney disease

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Optimizing outcomes for children with immune-mediated chronic kidney disease

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Editorial: Optimizing outcomes for children with immune-mediated chronic kidney disease

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KEYWORDS

immune-mediated, kidney disease, glomerular disease, CKD—chronic kidney disease, pediatrics

Editorial on the Research Topic
Optimizing outcomes for children with immune-mediated chronic kidney disease

Kidney involvement is an important determinant of morbidity and mortality in systemic immune-mediated diseases. Acute kidney injury may transition into chronic kidney disease (CKD) ultimately resulting in kidney failure warranting kidney replacement therapy. The glomerulonephritides (GN) are a heterogeneous group of immune-mediated diseases characterized by glomerular inflammation and injury (1, 2). GN is the most frequent cause of kidney failure in young people, representing a major burden regarding long-term kidney outcomes (3). The different mechanisms of immune dysregulation affecting the kidney are complex and heterogeneous (Figure 1) (4). Auto-antibodies directed against renal antigens can cause direct kidney disease; whereas indirect kidney disease can be induced by systemic autoimmunity, e.g., immune complex formation, alternative pathway complement activation, and the formation of neutrophil extracellular traps. Although there are many pathways of immune-mediated kidney inflammation, pathways leading to CKD are more homogenous (4). A loss of immune homeostasis in the kidney leads to further recruitment of immune cells and damage accrual. An uncoordinated tissue repair subsequently leads to tissue fibrosis. Immune function is severely compromised in kidney failure, leading to a vicious cycle facilitating further kidney disease and damage.

Significant knowledge gaps exist regarding immune-mediated kidney disease, particularly glomerular diseases, which impact the management of these patients and can lead to detrimental outcomes. Serial kidney biopsies are often warranted to assess diagnosis, disease state (active inflammation vs. chronic injury), treatment response, and prognosis. Unfortunately, current histopathological techniques applied in routine practice sometimes fail to adequately differentiate between the distinct subtypes of

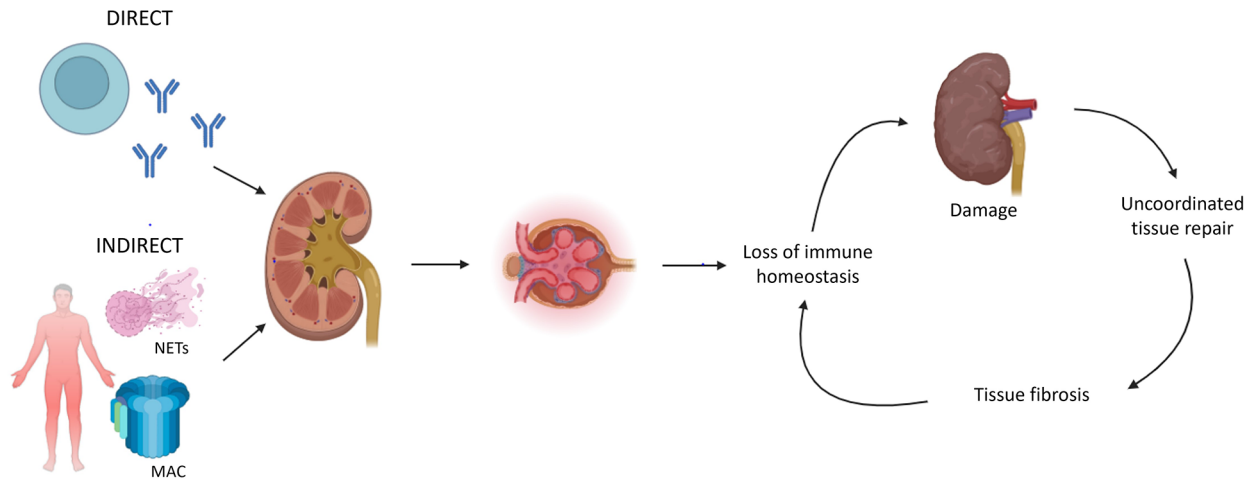

FIGURE 1
Key mechanisms of immune dysregulation leading to chronic kidney disease (CKD) (4). Immune-mediated kidney inflammation can be caused by direct (e.g., autoantibodies targeting renal antigens) or indirect (e.g., immune complex formation, complement activation, neutrophil extracellular trap formation) pathways. In both mechanisms, kidney inflammation subsequently leads to a loss of immune homeostasis, which facilitates further damage and the development of CKD. An uncoordinated tissue repair leads to fibrosis which compromises immune homeostasis. NETs = neutrophil extracellular traps; MAC = membrane attack complex (initiated by complement pathway activation).

glomerular diseases. There is a lack of widely-available, performant, and validated liquid (i.e., non-invasive) biomarkers that can inform treating physicians on disease-specific changes within the kidney. Most immune-mediated glomerular diseases are currently managed with broad-spectrum immunosuppressive therapies, which often cause side effects impacting patient quality of life, without adequate differentiation based on the underlying etiology. This approach underscores the critical need for the development of more targeted therapeutic options and the identification of biomarkers that can guide treatment decisions. Such advancements would enable personalized management strategies that could improve patient outcomes while minimizing adverse effects. Thus, the main objective of the current Research Topic was to report on and optimize long-term outcomes for children with immune-mediated kidney disease.

The burden of CKD in adolescents and young adults is underestimated. Sun et al. reported a significant increase in the global incidence of early-onset CKD in the past three decades. Nonetheless, the disability-adjusted life years rate remained stable, whereas mortality rates have decreased. Childhood-onset systemic-onset lupus erythematosus (cSLE) patients often exhibit more aggressive disease compared to adult patients, characterized by a higher incidence and more severe course of lupus nephritis (5, 6). The poor outcomes of cSLE patients are demonstrated in the study by Chen et al. Whereas up to 60.2% of the cSLE patients reached clinical remission during follow-up, only 3.5% reached complete remission (clinical and serological remission and immunosuppressant-free) and 19% reached steroid-free remission. Long-term remission was reached by only a minority of the patients. Crescentic GN encompasses a histopathological phenotype which can be observed in multiple GN subtypes, such as anti-neutrophil cytoplasmic antibodies (ANCA)-associated vasculitis (AAV). Zhang et al. compared outcomes of crescentic GN patients in the context of their etiology. Renal survival rates were lowest in patients with anti-GBM disease. These patients demonstrated more severe clinical manifestations and higher crescent scores on histopathology. AAV patients also had lower survival rates compared to the other GN subtypes presenting with crescents on histopathology. Borovitz et al. reported on C3 glomerulopathy relapse after kidney transplantation. In their case series of 19 C3 glomerulopathy patients, five underwent a kidney transplantation. Strikingly, all five patients experienced a relapse post-transplantation, which implies higher recurrence rates than previously believed. However, these results warrant validation in larger cohorts.

Three studies reporting on non-invasive biomarkers in glomerular diseases were incorporated in this Research Topic. In a prospective study by Zhaoyang et al. high pre-treatment serum levels of leptin and CCL22 were associated with steroid resistance in idiopathic nephrotic syndrome in childhood. Jiang et al. reported on vitamin D insufficiency in cSLE patients and its link with disease activity through changes in T helper 17 cells and regulatory T cells. Finally, Cody et al. tested the storage stability of six urinary biomarkers included in the Renal Activity Index for Lupus composite score.

The current Research Topic also encompasses two interesting case reports highlighting the difficulties in adequately discriminating between different GN subtypes. Kuang et al. reported the case of an eight-year-old girl with a myeloperoxidase-ANCA positive AAV initially presenting as a post-streptococcal acute GN. Daneshgar et al. described a case of C3 glomerulopathy relapse post-renal transplantation, complicated by a COVID-19 induced immune-complex mediated GN with membranoproliferative features and cryoglobulinemia.

Collectively, the papers incorporated in this Research Topic underscore the existing unmet needs in the diagnosis and

management of children with immune-mediated kidney disease. Notwithstanding long term kidney outcomes have improved over the past decades, the prognosis for these children remains poor. These studies may act as a starting point to further optimize outcomes for children with immune-mediated kidney disease, particularly those with glomerular diseases. Future research should prioritize the discovery of novel liquid and clinical biomarker profiles that can inform physicians on diagnosis, disease state, prognosis, and treatment response, as well as possible new therapeutic targets.

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Conflict of interest

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CASE REPORT
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Case Report: Clinical and Pathological Findings of a Recurrent C3 Glomerulopathy With Superimposed Membranoproliferative Glomerulonephritis Pattern and Cryoglobulinemia Associated With COVID-19

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Coronavirus disease 2019 (COVID-19) may cause a wide spectrum of kidney pathologies. The impact of COVID-19 is unclear in the context of the complement system abnormalities, including C3 glomerulopathy (C3G). In this report, we describe a young adult receiving a kidney transplant for C3 glomerulopathy (C3G), a disorder of the alternative complement pathway. The patient developed a recurrent C3G ∼7 months after transplantation. His post-transplant course was complicated by SARS-CoV-2 infection. There was a progression of glomerulonephritis, characterized by *de novo* immune-complex mediated membranoproliferative glomerulonephritis pattern of injury with crescentic and necrotizing features, along with positive immunoglobulins, persistent IgM staining and the presence of cryoglobulinemia. COVID-19 may have aggravated the inherent complement dysregulation and contributed to cryoglobulinemia observed in this patient. Our study of 5 sequential kidney allograft biopsy series implicates that COVID-19 in this patient promoted a superimposed immune complex-mediated glomerulonephritis with membranoproliferative glomerulonephritis (MPGN) pattern and cryoglobulinemia, which was a potentiating factor in allograft loss. This work represents the first report of cryoglobulinemic GN after COVID-19.

Keywords: COVID-19, glomerulonephropathy, cryoglobulinemia, case report, kidney transplant, membranoproliferative glomerulonephritis (MPGN), C3 glomerulopathy (C3G)

INTRODUCTION

Coronavirus disease 2019 (COVID-19) may lead to exaggerated activation of the complement system (1). The impact of COVID-19 is unclear among patients with abnormalities of the complement system, including C3 glomerulopathy (C3G). C3G is a rare disorder of the alternative complement pathway associated with progression to end-stage kidney disease (ESKD) and a high

risk for disease recurrence following kidney transplantation. We report the clinicopathological correlates for a kidney transplant recipient with a history of C3G who experienced COVID-19 amid post-transplant C3G recurrence.

CLINICOPATHOLOGICAL CORRELATION

A 19-year-old male with ESKD secondary to C3G underwent a deceased donor kidney transplant. His pre-transplant history was notable for the presence of serum C3 nephritic factor, which led to his complement dysregulation and C3G. The native kidney biopsy taken 2 years before the transplant was characterized by diffuse proliferative and crescentic glomerulonephritis with prominent C3 (3+) and negative immunoglobulins staining. Electron microscopy (EM) showed ribbon-like dense transformation of the lamina densa of the glomerular basement membrane (GBM) consistent with a dense deposit disease (DDD) (**Figure 1A**).

His post-transplant course was uneventful, with a nadir serum creatinine of 1.3 mg/dL within 2 weeks of transplant. Maintenance immunosuppressants included tacrolimus 1 mg BID with a therapeutic goal of 3–5 ng/mL and mycophenolate (MMF) at 500 mg BID. Due to elevated serum creatinine (1.8 mg/dL), a kidney biopsy (#1) was performed 1-month post-transplant which showed mild acute tubular injury as the only pathological finding. There was no evidence of rejection or disease recurrence (**Figure 1B**) with negative immunofluorescence (IF) studies and no evidence of deposits on EM (**Figure 2A**). Thus, maintenance immunosuppressants were continued without any adjustment. Approximately 7 months post-transplant, kidney biopsy (#2) was performed due to new-onset microscopic hematuria (56 RBCs/HPF) and sub-nephrotic proteinuria (2.48 by UPCR), which demonstrated early recurrent C3G characterized by mild mesangial and endocapillary hypercellularity; mild glomerular staining for C3 (1+) but negative for other Immunoglobulins (**Figure 1C**); and numerous intramembranous and mesangial electron-dense deposits on EM (**Figure 2B**). His serum C3 was decreased (50 mg/dL). The patient was started on a new complement inhibitor study drug, iptacopan the first dose at 10 mg BID (4/1/2020), with weekly dose escalation to 50 mg BID, 100 mg BID, then to full dose at 200 mg BID (4/21/20). This full dose was maintained for ∼11 months. Iptacopan is a factor B inhibitor that blocks the activation of the alternative complement pathway. His renal function was stable after iptacopan treatment. At ∼14 months post-transplant, he developed nephrotic range proteinuria, significant microscopic hematuria with a serum creatinine of 2 mg/dL. A kidney biopsy (#3) performed at that time demonstrated mesangial and endocapillary hypercellularity with accentuated lobulation and segmental duplication of the GBM (**Figure 1D**). Additional findings included mild tubulitis (t1) and lymphocytic infiltrates in the interstitium (i1), suggestive of Banff borderline acute cellular rejection (T-cell mediated rejection), for which he received 2-week steroids, including a pulse methylprednisolone (**Figure 1D**). Immunofluorescence studies showed prominent glomerular C3 staining (3+). EM showed electron-dense deposits in the subendothelial, mesangial, and intramembranous regions. Segmental GBM showed elongated intramembranous deposits in a ribbon-like distribution, although dark osmiophilic deposits, characteristics of DDD, were not identified. Rare duplication of GBM with cell interposition was noted (**Figure 2C**). The tubuloreticular inclusions were not identified. These glomerular findings were consistent with a recurrent C3G. Examination of donor specific antibodies (DSA) was negative, excluding the diagnosis of antibody mediated rejection.

Two months later (16 months post-transplant), the patient presented with worsening peripheral edema, hypoalbuminemia, hypertension, nephrotic range proteinuria, microscopic hematuria, and a rapid rise in serum creatinine level from 1.6 to 3.2 mg/dL. There was no fever, diarrhea, myalgia, respiratory symptoms, or loss of taste / smell. As COVID-19 testing is routinely performed during the pandemic, the patient was incidentally tested positive for COVID-19 by PCR upon admission. Kidney biopsy (#4) showed cellular crescents (2/13 glomeruli, ∼15%) and fibrocellular crescents (5/13 glomeruli, ∼23%). While the intensity of glomerular C3 staining decreased to 1–2+ (**Figure 1E**), there were new findings of glomerular IgG (3+), IgM (3+), IgA (2+), and C1q (trace) staining. EM showed numerous electron-dense deposits in the subendothelial, intramembranous, and mesangial areas. Some of these deposits had a repetitive pattern of vague, short, organized fibril substructures (**Figures 2D,E**). Tubuloreticular inclusions were now found in some endothelial cells (**Figure 2F**). These findings supported a "full-house" immune-complex mediated glomerulonephritis (IC-GN) with features of MPGN and crescents formation. There was also borderline acute T-cell mediated rejection by Banff criteria, for which he received another 2-week steroids, including a pulse methylprednisolone.

The patient's kidney function worsened (serum creatinine 4.48 mg/dL) with heavy proteinuria and hematuria (**Figure 1**). He subsequently developed low C4 (< 5 mg/dL). His C3 remained slightly below normal at 80–85 mg/dL. A fifth kidney biopsy (#5) at 19 months post-transplant demonstrated diffuse fibrocellular (9/23 glomeruli, 39%) and fibrous crescentic lesions (6/23, 26%). The remaining glomeruli showed MPGN pattern (**Figure 1F**) with the progression of interstitial fibrosis and tubular atrophy (20–25%). There was acute tubular injury, moderate tubulitis (t2), and mild interstitial inflammation (i1). Immunofluorescence showed decreased staining intensity for IgG (3+ to 1+) and C3 (2+ to 1+) but persistent IgM staining (3+), with the pattern of granular, predominantly capillary walls staining, which was also positive for Kappa (3+) and Lambda (2+) (**Figures 2M,N**). EM showed electron-dense deposits in the intramembranous, mesangial, and subendothelial regions (**Figures 2G,H**).

Additional laboratory evaluations showed negative ANCA, myeloperoxidase and proteinase 3 antibodies, double-stranded DNA, and antinuclear antibodies (ANA) but positive cryoglobulins (5 mg/dL). He was not receiving any medications known to cause lupus-like nephritis. A thorough evaluation for other infectious etiologies, including viral hepatitis panels was negative. IHC studies for CD68, a marker of macrophages,

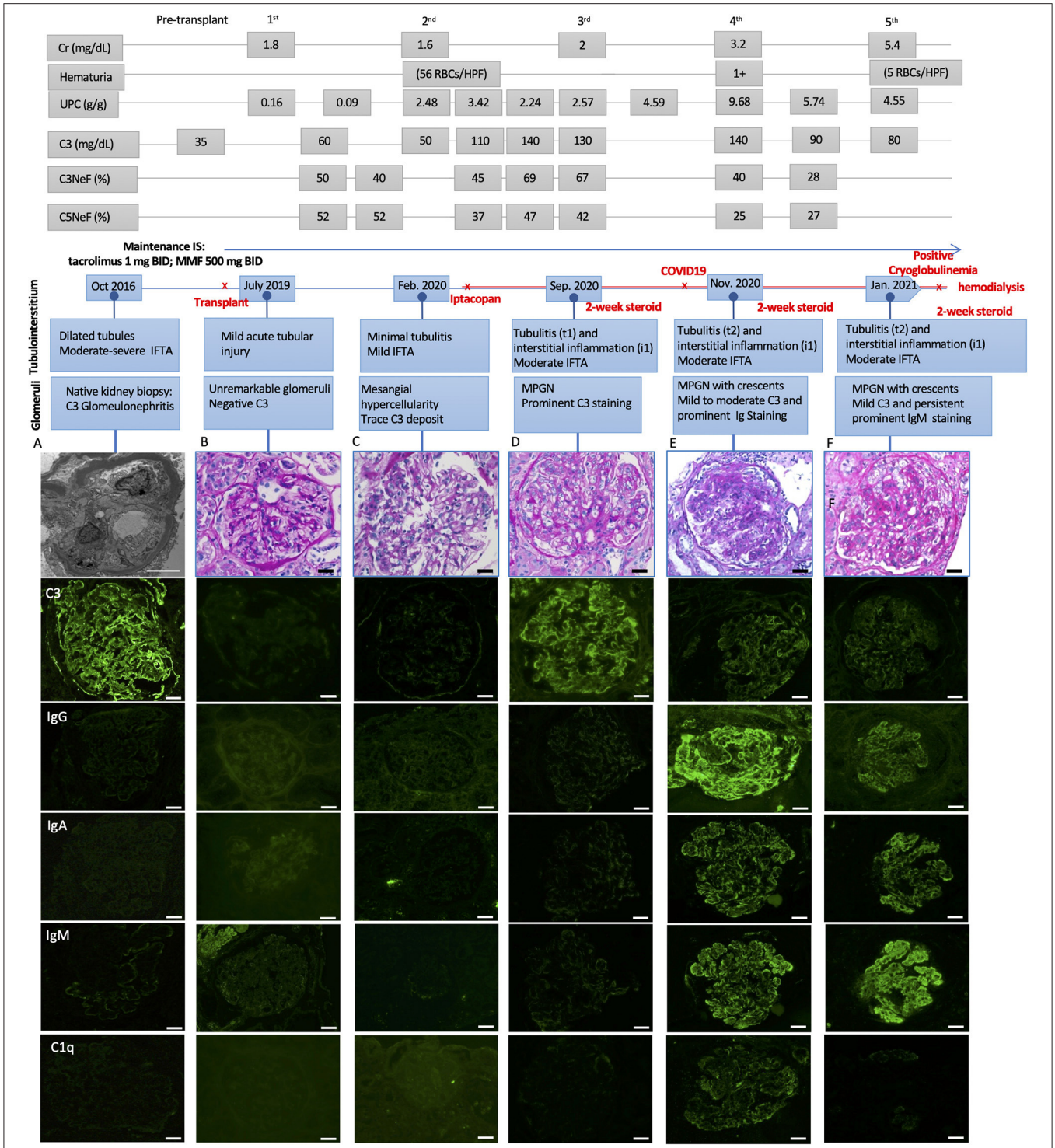

FIGURE 1 | Light microscopic and immunofluorescence findings in a patient with C3G recurrence and COVID-19 infection with respective laboratory results, including serum creatinine (Cr), hematuria, urine protein creatinine ratio (UPC), C3, C3, and C5 Nephritic factors (Normal ranges: C3 > 85 mg/dL, C3Nef < 20%, C5Nef < 20%). **(A)** The native kidney biopsy showing intramembranous band-like electron dense deposition under electron microscope with prominent C3 staining (3+) and negative immunoglobulins staining. **(B)** The first biopsy 4 weeks after deceased-donor renal transplantation showing unremarkable glomeruli with negative immunofluorescence staining and mild acute tubular injury. **(C)** The second biopsy showing mild mesangial and focal endocapillary hypercellularity with mild glomerular C3 staining. **(D)** The third biopsy showing focal membranoproliferative glomerulitis (MPGN) lesion with prominent C3 staining (3+) and negative immunoglobulins staining. There was mild tubulointerstitial inflammation, suggestive of Banff borderline T-cell mediated rejection, for which 2-week steroid was given.

(Continued)

FIGURE 1 | (E) The fourth biopsy showing MPGN pattern with focal crescentic lesions, mild to moderate C3 staining (1–2+), prominent immunoglobulins staining, including IgG (3+), IgA (2+) and IgM (3+), and mild C1q staining (trace-1+). There was mild to moderate tubulointerstitial inflammation, suggestive of Banff borderline T-cell mediated rejection, for which 2-week steroid was given. **(F)** The fifth biopsy showing MPGN pattern with diffuse crescentic lesions, mild C3 staining (1+) and persistent prominent IgM staining (3+), mild to moderate IgG and IgA (1–2+), negative C1q staining. There was mild to moderate tubulointerstitial inflammation, suggestive of Banff borderline T-cell mediated rejection, for which another 2-week steroid was given. Scale bar: 50 μm, EM scale bar: 5 μm.

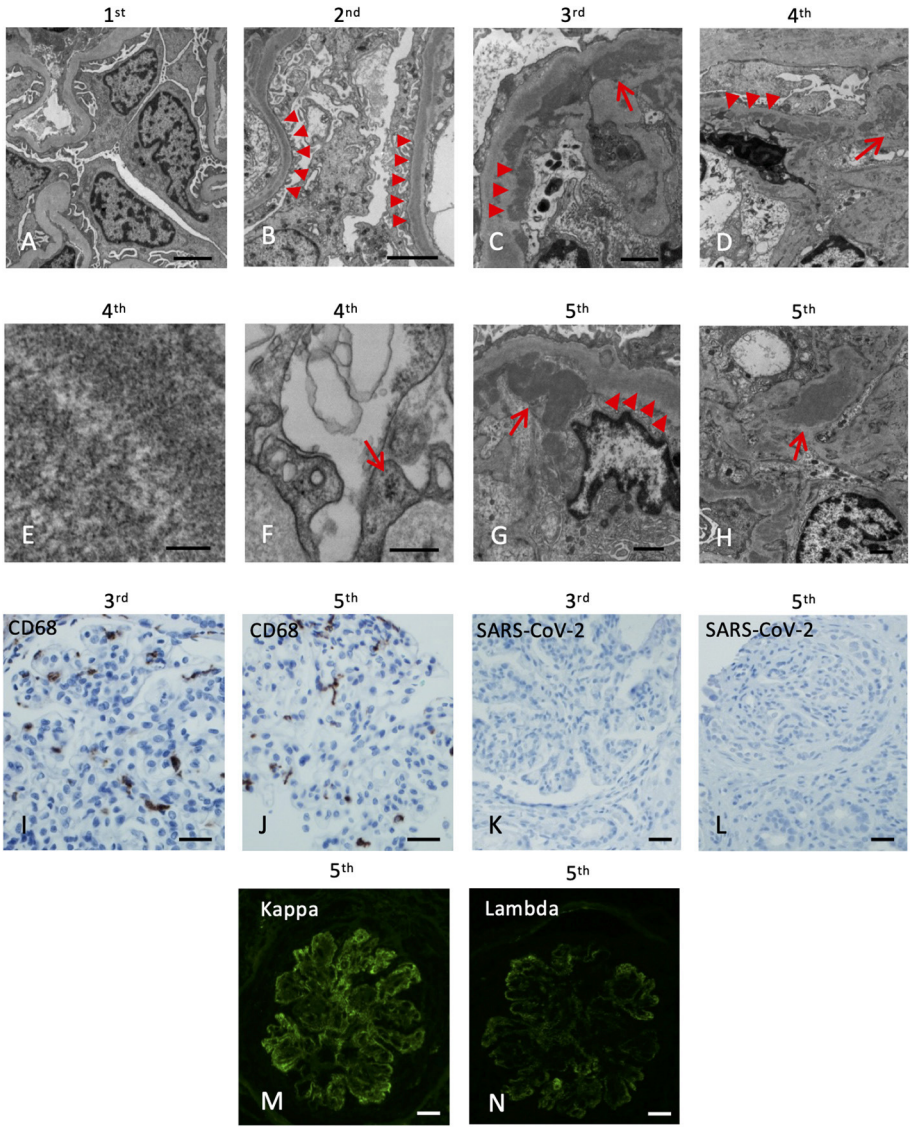

FIGURE 2 | Electron microscopy and immunohistochemistry of the five kidney allograft biopsies. The labels represent the sequence of the biopsies. **(A)** Electron microscopy of the 1st renal biopsy showing no electron-dense deposition (Scale bar: 2 um). **(B)** Electron microscopy of the 2nd renal biopsy showing intramembranous electron-dense deposits (arrowheads, Scale bar: 2 um). **(C)** Subendothelial (arrowheads) and mesangial (arrow) electron-dense deposits (Scale bar: 1 um). **(D)** Abundant depositions in the intramembranous (arrowheads) and the paramesangial area (arrow) of the 4th renal biopsy (arrow) (Scale bar: 2 um). **(E)** High magnification revealed the deposits composed of vague, short, organized "wormy" fibrils substructure (Scale bar: 100 nm). **(F)** Tubuloreticular inclusions in the endothelium (Scale bar: 300 nm). **(G)** Abundant depositions in the intramembranous (arrowheads) and the subendothelial areas (arrow) of the 5th renal biopsy (Scale bar: 1 um). **(H)** Mesangial depositions in the 5th renal biopsy (arrow). (Scale bar: 1 um). **(I,J)** The CD68 immunostaining of the 3rd and 5th renal biopsy revealed increase histiocytic infiltrates in the glomeruli. **(K,L)** Immunohistochemical staining of SARS-CoV-2 nucleocapsid protein of the 3rd and 5th renal biopsy are both negative (Scale bar: 50 um). **(M,N)** Immunofluorescence of the 5th biopsy shows positive Kappa (3+) and Lambda (2+) staining (Scale bar: 50 um).

highlighted infiltration of macrophages in the glomeruli in biopsy #3 (**Figure 2I**) and #5 (**Figure 2J**). IHC studies using anti-SARS-CoV-2 Nucleocapsid protein (performed on 3rd to 5th biopsies; no tissue left from 4th biopsy) were negative, and no viral-like particles were identified by EM (**Figures 2K,L**). Taken together, the last two biopsies demonstrated MPGN pattern

of injury with several features suggestive of cryoglobulinemic glomerulonephritis. After ∼22 months post-transplant, despite normalization of serum C3 and improvement in serum C3Nef and C5Nef, his allograft progressed to ESKD.

DISCUSSION

We described a kidney transplant patient with early recurrence of C3G, later superimposed with immune complex-mediated MPGN and cryoglobulinemia following SARS-CoV-2 infection. C3G is caused by dysregulation of the alternative complement pathway (2). The overall kidney prognosis is poor. Most C3G will progress to end-stage kidney disease (3), with a high risk for disease recurrence (∼50–80%) causing allograft failure after transplantation (4).

Our patient developed C3G recurrence at ∼7 months post-transplant. The normalization of serum C3 levels and the decrease in the intensity of glomerular C3 staining in the allograft after iptacopan suggests a gradual remission of the complement abnormalities. His clinical course was complicated by COVID-19 infection. Following COVID-19, he was noted to have hypocomplementemia with low C4 (<5 mg/dL) and borderline low C3 (83 mg/dL). The last two biopsies demonstrated an immune-complex mediated glomerulonephritis with MPGN and crescentic lesions, characterized by strong immunoglobulin M and immunoglobulin G staining. The persistent IgM staining (biopsy #5), presence of substructures within electron-dense deposits, and the progression of MPGN despite improvement of C3 argue against C3G as the main driver of worsened kidney function. Rather, these findings suggest a superimposed immune-complex mediated glomerulonephritis as the catalyst for deteriorating kidney function. Laboratory examination after COVID-19 infection showed positive cryoglobulinemia, concurrent with the deterioration of serum creatinine, progressive nephrotic range proteinuria and hematuria.

Kidney involvement has been reported in COVID-19, ranging from 17 to 56.9% of patients (5, 6). Common pathological findings in COVID-19 related nephropathy include acute tubular injury (7), collapsing glomerulopathy and thrombotic microangiopathy (8). Findings of crescentic glomerulonephritis, including ANCA-vasculitis and anti-GBM disease, and immune complex glomerulonephritis with full house immunofluorescence have been reported in rare cases of COVID-19 (8–10). The sensitivity and specificity of SARS-CoV-2 detection in kidney tissues are not well-established, with the controversial interpretation of "viral-like particles" identified by EM (11–13). In these post-COVID-19 kidney biopsies, IHC studies using anti-SARS-CoV-2 Nucleocapsid protein and attempts to identify viral particles by EM were both negative.

We hypothesize that COVID-19 associated cryoglobulinemia and membranoproliferative pattern of glomerulonephritis accelerated the progression of allograft dysfunction in this patient with early C3G recurrence. The repetitive substructures of electron-dense deposits in the GBM, subendothelial areas, and mesangium resembled the so-called "short wormy" substructure reported in cryoglobulinemic glomerulonephritis (14). Cryoglobulinemic GN is well-known to be associated with viral infections (such as hepatitis C or B virus), autoimmune diseases, and lymphoma (15, 16). All of these conditions (other than COVID-19) were absent in this patient. Tubuloreticular inclusions, a marker of increase in systemic interferons, were not present in biopsy #3 but were subsequently noted in biopsy #4 following COVID-19, which is well-known to associate with a cytokine surge (17). To the best of our knowledge, this is the first report of cryoglobulinemic GN after SARS-CoV-2 infection.

The potential impact of inherent complement dysregulation in the setting of COVID-19 infection cannot be understated. COVID-19 has been shown to activate complement pathways (1) which can further aggravate the underlying complement dysregulation of our patient. A previous study on a patient with relapsed atypical hemolytic uremic syndrome (HUS) after COVID-19 supports this notion (18). IC-GN in the setting of COVID-19 has been recently reported in a patient with HUS (10). Since a "full-house" IC-GN with diffuse electron-dense deposits in mesangium is rare in HUS, this feature was more likely associated with COVID-19(10). Our patient experienced early, recurrent C3G and later developed a superimposed IC-MPGN with features of cryoglobulinemic GN following SARS-CoV-2 infection (**Figure 1**) without clinical or pathological features of HUS, or antibody mediated rejection. Our report suggests that COVID-19 infection could be an additional, significant risk factor for allograft loss in transplant recipients with inherent complement dysregulation.

The strength of our study include a thorough follow up of the patient with 5 serial kidney biopsies and laboratory workups. However, given the complexity of his clinical course, proving that COVID-19 is the direct cause of immune complex-mediated glomerulonephritis with membranoproliferative glomerulonephritis (MPGN) pattern and cryoglobulinemia was not possible, although many other etiologies of cryoglobulinemia and MPGN were excluded.

PATIENT PERSPECTIVE AND CONSENT

The patient provided verbal consent to have his story shared in this manner in hopes that it would improve care for others.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

ND, P-IL, LH, and D-FD performed renopathological study and manuscript writing. CN provided

critical revision and editing of the manuscript. All authors contributed to the article and approved the submitted version.

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Stability of novel urinary biomarkers used for lupus nephritis

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Background: The Renal Activity Index for Lupus (RAIL) is a composite score of six urinary biomarkers (neutrophil gelatinase–associated lipocalin (NGAL), monocyte chemoattractant protein-1 (MCP-1), kidney injury molecule-1 (KIM-1), ceruloplasmin, adiponectin, and hemopexin) used to monitor lupus nephritis activity in children. We tested stability of RAIL biomarkers prior to meaningful clinical use.

Methods: Urine samples were tested by ELISA under shipping conditions, freeze/thaw, ambient and longer-term storage. Statistical analysis was performed *via* Deming Regression, Bland-Altman and Spearman Correlation Coefficient.

Results: Biomarker concentration were comparable to freshly collected urine following storage at −80 °C for up to 3 months, and at 4 or 25 °C up to 48 h followed by −80 °C. Neither shipping on dry or wet ice exposure nor addition of two freeze-thaw cycles led to loss of signal, with excellent Spearman Correlation coefficients under all conditions.

Conclusions: RAIL biomarkers are stable following short-term storage at clinically relevant conditions.

KEYWORDS

lupus nephritis, urine, biomarker, stability, SLE

Introduction

Lupus nephritis (LN) confers a poor prognosis, as 5–25% of patients with proliferative LN develop kidney disease within 5 years of diagnosis, and about 10% of all patients with LN will develop end-stage kidney disease (1, 2). Disease with onset in childhood or adolescence is termed childhood onset SLE, or cSLE. Outcomes for cSLE are worse than that of adults and children have higher occurrence of LN (3–5). Current laboratory testing is inadequate to diagnose LN activity effectively and evaluate response to therapy, which makes evaluating potential new therapies challenging (6, 7). In fact, traditional laboratory measures will classify patients incorrectly 30–40% of the time when compared to kidney biopsy. As such, complement levels and anti-dsDNA antibody titers

are not effective for predicting the course of LN (6, 8, 9). Therefore, research has focused on developing new laboratory tests to non-invasively, accurately, and rapidly diagnose LN and detect its response to treatment.

We have previously described and validated a panel of six urinary biomarkers, neutrophil gelatinase-associated lipocalin (NGAL), monocyte chemoattractant protein-1 (MCP-1/CCL2), kidney injury molecule-1 (KIM-1), ceruloplasmin, adiponectin, and hemopexin. Considering the urinary concentration of all six biomarkers, the pediatric Renal Activity Index for Lupus (RAIL) can be calculated, where a higher score reflects high renal inflammation, and the score is >90% accurate in detecting LN activity histologic activity measures (10–12). The standard operating procedures for collecting and storing urine specimens to be used for RAIL biomarker measurement has yet to be established. We have previously demonstrated the short- and long-term of NGAL and KIM-1 in pediatric AKI patients. We found no significant difference between concentration measurements at baseline (immediate testing within 15 min of aliquoting), 24 h at 4 °C, or 24 h at −80 °C (13). In addition, NGAL and KIM-1 demonstrated long-term stability, without relevant degradation at long term storage, for up to 5 years. To further move the RAIL biomarkers toward clinical utility, we conducted stability testing for the remaining four urinary biomarkers that make up the RAIL score under common clinical and experimental conditions. We hypothesized that the markers would be stable under various clinically relevant conditions including shipping, temperatures, and longer storage.

Materials and methods

Patients and samples

Patients enrolled in the SLE Clinical and Research Database were eligible for the study (IRB CCHMC-2008-0635). Patients were recruited over a 1-month period in September 2021 from Nephrology and Rheumatology clinics. Inclusion criteria were diagnosis of SLE by EULAR/ACR 2019 criteria (14) with or without nephritis. There was no requirement based on disease activity. Exclusion criteria were diagnosis of kidney disease other than Lupus Nephritis, diagnosis of autoimmune disease besides SLE, urine volumes below 10 ml or patient unwilling to provide urine.

A random urine sample was collected from each patient and processed within 2 h of collection. Urine was centrifuged for 10 min at 2200x g prior to aliquoting for testing the 10 experimental conditions, as shown in Figure 1. Control condition was freezing at −80 °C and run within 1 week of collection. All thaw times were 45 min.

Urine biomarker assays

Unless stated otherwise, biomarkers were quantified using commercial ELISA kits as per the manufacturer's instructions. MCP-1 used a four-parameter logistic curve to fit the standard curve. Adiponectin, Ceruloplasmin, and Hemopexin used a log/log curve to fit the standard curve.

Human MCP-1 was measured *via* ELISA (R&D Systems, Minneapolis, MN, DCP00), diluted 1:1, with a mean minimal detectable dose of 1.7 pg/mL. Intra-assay and inter-assay were 5% and 5.1%, respectively.

Human Adiponectin was measured by commercially available ELISA kit (R&D system, Minneapolis, MN, DRP300), diluted 1:5, with a mean minimal detectable dose of 0.246 ng/ml. Intra-assay and inter-assay coefficients of variation were 3.7 and 6.8%, respectively.

Human Ceruloplasmin was measured by ELISA Kit (Assaypro LLC, St. Charles, MO, EC4201-1), diluted 1:50, with a mean minimal detectable dose of 0.085 ng/ml. Intra-assay and inter-assay were 4.9 and 9.8%, respectively.

Human Hemopexin was measured by commercially available ELISA kit (Assaypro LLC, St. Charles, MO, EH2001-1), diluted 1:20, with a mean minimal detectable dose of 4.2 ng/ml. Intra-assay and inter-assay coefficients of variation were 4.7 and 9.2%, respectively.

Statistical analysis

Natural log-transformation of each biomarker was performed prior to analysis to ensure normal distribution assumptions. Analyte concentrations that were above or below the limits of detection were imputed by 50% of the level of lower limit of detection (LLD) and 50% over the upper limit of detection (ULD), respectively. Means were calculated for each biomarker under each condition. Spearman correlation coefficients were calculated for each experimental condition with the control condition, with of 0.1–0.39 representing weak correlation, 0.4–0.69 representing moderate correlation, 0.7–0.89 representing strong correlation, and >0.9 representing very strong correlation (15). Conditions were then compared to baseline using Deming regression and Bland-Altman bias plots. Since the urinary biomarkers measured under different experimental conditions (including the control condition) may be subject to some degree of measurement variabilities, we took Deming Regression approach, modeling the relationship between each pair of experimental conditions with the control condition. For the Bland-Altman plots, the differences between the experimental vs. control condition for a given urinary biomarker are shown in the y-axis while the corresponding measurement under the control condition is shown in the x-axis. For each urinary biomarker the mean difference, or bias,

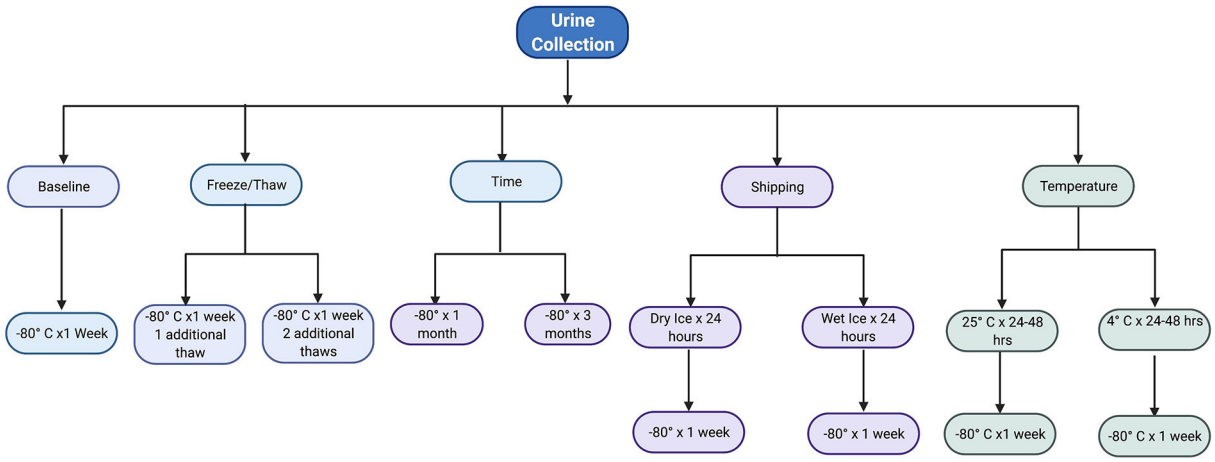

FIGURE 1
Flowchart of the 10 experimental conditions per urine sample. Image made with BioRender.

and the 95% limits of agreement was displayed (16). When the majority of measurements were within the 95% limits of agreement, then a given biomarker was considered stable across the tested experimental conditions. For month 1 and month 3 samples, all urinary biomarkers were repeated four times, so intraclass correlation coefficients (ICC) and coefficients of variation (CV) were calculated. An ICC of < 0.5 represents poor reliability, between 0.5 and 0.75 represent moderate reliability and 0.75 to 0.9 indicates good reliability, and >0.9 indicates excellent reliability (17). All statistical analyses were performed using SAS 9.4 (Cary, NC).

Results

Patient urine specimens were obtained in a serial fashion, resulting in 10 urine samples from 10 patients. The average age of the patient population was 19.09 ($\pm$ 2.51) years. There were 9 females (90%), and the majority were Caucasian (70%), with the remaining African American. Most of the patients (80%) did not have a diagnosis of lupus nephritis, and the two that did have Class II Lupus Nephritis. The urine volumes were sufficient that each collection was able to be run under each experimental collection. MCP-1 had the least number of samples below the lower limit of detection, only 1/220 (0.45%). Adiponectin had 44/220 (20%) and Hemopexin had 25/220 (11.36%) below the lower limit of detection. Ceruloplasmin has samples both above and below the limits of detection, each 44/220 (20%).

Adiponectin

The means of adiponectin under baseline condition was 1.935 $\pm$ 1.521, with minimum of -0.486 and maximum of 3.441 from the 10 samples included. As shown in Table 1, the log-transformed means for experimental conditions ranged from 1.626 $\pm$ 1.482 (average 15.97% decrease, after 3-month storage) to 2.132 $\pm$ 1.476 (under Fridge condition). Spearman correlation coefficients showed duplicates had very good correlation within each sample run, all >0.9, except for room temperature, which was good at 0.888. The Bland-Altman plots (Figure 2) and Deming-Regression (Table 2) show that differences in means for each experimental condition are due to random error. Finally, Table 3 shows ICC for 1 month and 3 months storage conditions, revealing excellent reliability.

Ceruloplasmin

Likewise, mean for ceruloplasmin under baseline condition was 3.618 $\pm$ 2.248, with minimum of 0.754 and maximum of 6.669 from the 10 samples. As shown in Table 1, the log-transformed means for experimental conditions ranged from 3.483 $\pm$ 2.25 (average 3.73% decrease, under Fridge condition) to 4.245 $\pm$ 1.538 (after 3-month storage). Spearman correlation coefficients showed duplicates had very good correlation within each sample run, all >0.9 apart from the first freeze thaw experiment. The Bland-Altman plots and Deming-Regression are shown in Figure 3 and Table 2 respectively. There was some variability within the lower limits of detection but overall, differences in means for each experimental condition are due to random error. Finally, Table 3 shows ICC for 1 month and 3 months storage conditions, revealing excellent reliability.

Hemopexin

Mean for hemopexin under baseline condition was 5.522 $\pm$ 0.835, with minimum of 3.738 and maximum of 6.575

TABLE 1 Average urinary biomarker concentration under each experimental condition with spearman correlation coefficients.

	Condition	Mean	St dev	Min	Max	Spearman correlation coefficient	*P*-value
Adiponectin	Baseline	1.935	1.521	−0.486	3.441	0.973	<0.01
	Dry ice	1.901	1.466	−0.486	3.438	0.979	<0.01
	Wet ice	2.045	1.530	−0.486	3.681	0.961	<0.01
	Fridge	2.132	1.476	−0.486	3.626	1.00	<0.01
	RT	2.025	1.530	−0.018	3.615	0.888	<0.01
	FT1	1.862	1.627	−0.486	3.469	0.998	<0.01
	FT2	1.976	1.596	−0.486	3.483	0.988	<0.01
	1MO	1.950	1.532	−0.486	3.478	0.982	<0.01
	3MO	1.626	1.482	−0.486	3.220	0.910	<0.01
Ceruloplasmin	Baseline	3.618	2.248	0.754	6.669	0.991	<0.01
	Dry-ice	3.552	2.180	0.754	6.669	1.00	<0.01
	Wet ice	3.553	2.305	0.754	6.669	1.00	<0.01
	Fridge	3.483	2.250	0.754	6.669	0.984	<0.01
	RT	3.614	2.175	0.754	6.669	1.00	<0.01
	FT1	3.692	2.310	0.754	6.669	0.869	<0.01
	FT2	3.491	2.326	0.754	6.669	1.00	<0.01
	1MO	3.803	2.071	0.754	6.669	1.00	<0.01
	3MO	4.245	1.538	2.259	6.669	0.962	<0.01
Hemopexin	Baseline	5.522	0.835	3.738	6.575	0.915	<0.01
	Dry ice	5.524	0.887	3.738	6.643	0.989	<0.01
	Wet ice	5.530	0.847	3.738	6.663	0.976	<0.01
	Fridge	5.510	0.862	3.738	6.741	0.964	<0.01
	RT	5.413	1.004	3.738	6.663	0.963	<0.01
	FT1	5.488	0.935	3.738	6.602	0.961	<0.01
	FT2	5.470	0.898	3.738	6.533	0.967	<0.01
	1MO	5.536	0.905	3.738	6.712	0.973	<0.01
	3MO	5.817	0.897	4.187	6.897	0.952	<0.01
MCP-1	Baseline	4.558	1.600	1.760	6.448	1.00	<0.01
	Dry ice	4.596	1.562	2.255	6.379	1.00	<0.01
	Wet ice	4.556	1.633	1.888	6.468	1.00	<0.01
	Fridge	4.503	1.710	1.386	6.397	1.00	<0.01
	RT	4.426	1.813	0.987	6.423	0.999	<0.01
	FT1	4.553	1.582	2.135	6.376	0.993	<0.01
	FT2	4.471	1.751	1.456	6.408	1.00	<0.01
	1MO	4.673	1.504	2.309	6.402	0.992	<0.01
	3MO	4.607	1.601	2.156	6.467	1.00	<0.01

from the 10 samples. As shown in Table 1, the log-transformed means for experimental conditions ranged from 5.413 ± 1.004 (average 1.8% decrease, under Room Temperature) to 5.817 ± 0.897 (after 3-month storage). Spearman correlation coefficients showed duplicates had very good correlation within each sample run, all >0.9. The Bland-Altman plots (Figure 4) and Deming-Regression (Table 2) show that differences in means for each experimental condition are due to random error. Finally, Table 3 shows ICC for 1 month and 3 months storage conditions, revealing excellent reliability.

MCP-1

Mean of MCP-1 was 4.558 ± 1.600, with minimum of 1.760 and maximum of 6.448 from the 10 samples at baseline condition. As shown in Table 1, the log-transformed means for experimental conditions ranged from 4.426 ± 1.813 (average 2.90% decrease, under Room Temperature) to 4.673 ± 1.504 (after 1-month storage). Spearman correlation coefficients showed duplicates had very good correlation within each sample run, all >0.9. The Bland-Altman plots (Figure 5) and

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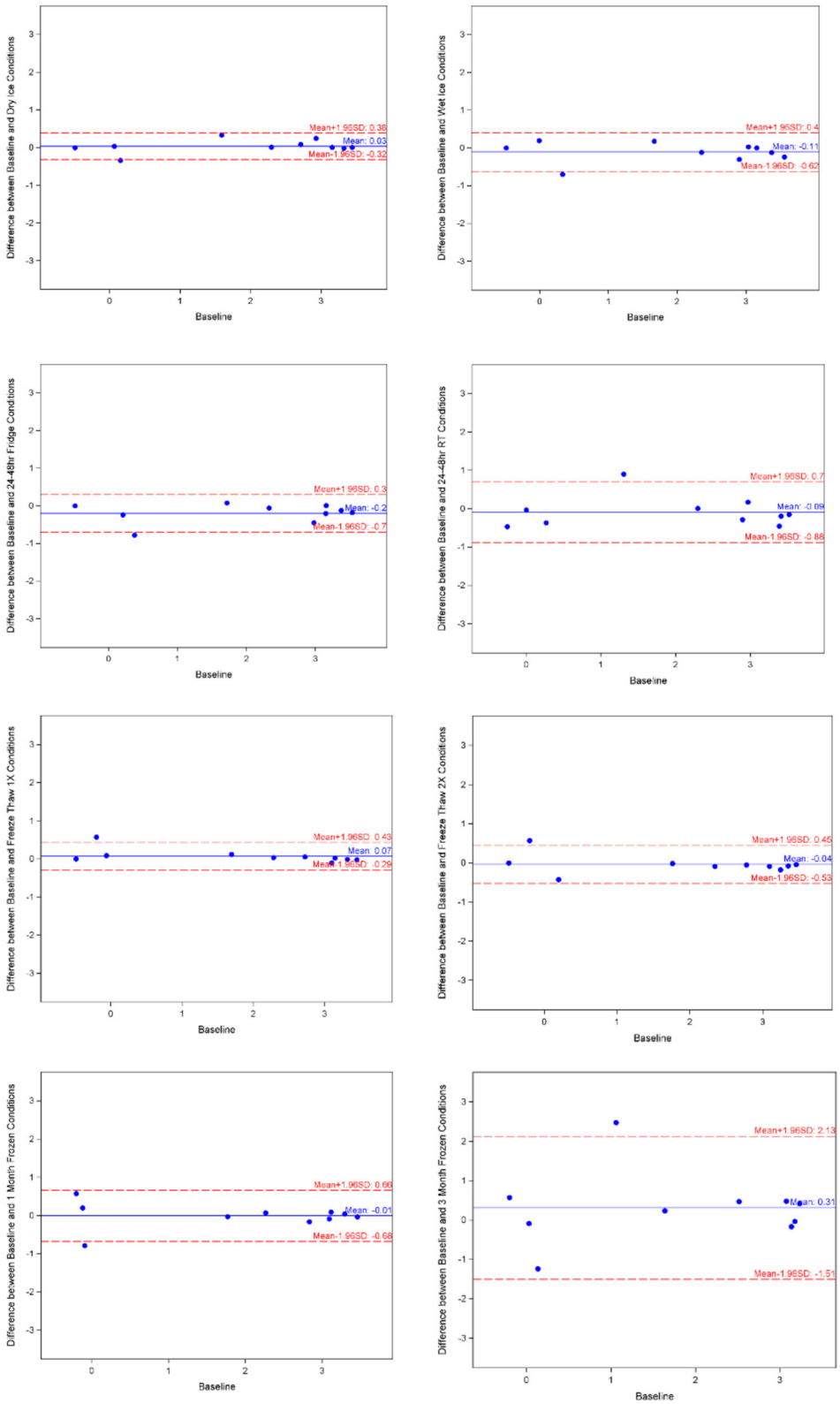

FIGURE 2
Adiponectin Bland-Altman charts by experimental condition. Image made with BioRender.

TABLE 2 Deming-Regression Intercept and beta-coefficient for each biomarker under each experimental condition.

	Dry ice	Wet ice	Fridge	Room temperature	Freeze thaw 1X	Freeze thaw 2X	1 month frozen	3 month frozen
Adiponectin	0.04	0.1	0.26	0.08	−0.21	−0.06	0	−0.24
	(95% CL −0.24, 0.32)	(95% CL −0.44, 0.64)	(95% CL −0.28, 0.79)	(95% CL −0.56, 0.72)	(95% CL −0.64, 0.22)	(95% CL −0.67, 0.56)	(95% CL −0.96, 0.96)	(95% CL −2.09, 1.62)
	0.96	1.01	0.97	1.01	1.07	1.05	1.01	0.96
	(95% CL 0.87, 1.06)	(95% CL 0.83, 1.18)	(95% CL 0.79, 1.15)	(95% CL 0.8, 1.22)	(95% CL 0.92, 1.22)	(95% CL 0.84, 1.26)	(95% CL 0.68, 1.34)	(95% CL 0.34, 1.59)
Ceruloplasmin	0.05	−0.16	−0.14	0.11	−0.03	−0.25	0.56	1.85
	(95% CL −0.53, 0.63)	(95% CL −0.45, 0.13)	(95% CL −0.44, 0.16)	(95% CL −0.41, 0.64)	(95% CL −0.36, 0.3)	(95% CL −0.66, 0.15)	(95% CL −2.2, 3.31)	(95% CL 0.79, 2.92)
	0.97	1.03	1	0.97	1.03	1.04	0.9	0.66
	(95% CL 0.86, 1.08)	(95% CL 0.97, 1.08)	(95% CL 0.93, 1.07)	(95% CL 0.87, 1.06)	(95% CL 0.93, 1.12)	(95% CL 0.96, 1.11)	(95% CL 0.38, 1.41)	(95% CL 0.43, 0.89)
Hemopexin	−0.35	−0.07	−0.19	−1.39	−0.74	−0.49	−0.46	−0.22
	(95% CL −1.4, 0.71)	(95% CL −0.5, 0.36)	(95% CL −1.17, 0.8)	(95% CL −6.45, 3.68)	(95% CL −3.56, 2.08)	(95% CL −2.68, 1.69)	(95% CL −2, 1.07)	(95% CL −6.46, 6.02)
	1.06	1.01	1.03	1.23	1.13	1.08	1.09	1.09
	(95% CL 0.88, 1.24)	(95% CL 0.93, 1.1)	(95% CL 0.84, 1.22)	(95% CL 0.38, 2.09)	(95% CL 0.65, 1.6)	(95% CL 0.72, 1.44)	(95% CL 0.81, 1.36)	(95% CL 0.04, 2.15)
MCP-1	0.15	−0.1	−0.37	−0.75	0.05	−0.52	0.39	0.04
	(95% CL −0.85, 1.14)	(95% CL −0.62, 0.42)	(95% CL −0.86, 0.12)	(95% CL −1.53, 0.04)	(95% CL −0.85, 0.94)	(95% CL −0.75, −0.29)	(95% CL −0.37, 1.15)	(95% CL −0.87, 0.96)
	0.98	1.02	1.07	1.13	0.99	1.09	0.94	1
	(95% CL 0.79, 1.16)	(95% CL 0.92, 1.12)	(95% CL 0.98, 1.16)	(95% CL 0.99, 1.28)	(95% CL 0.82, 1.15)	(95% CL 1.04, 1.15)	(95% CL 0.8, 1.08)	(95% CL 0.83, 1.17)

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TABLE 3 ICC values at 1- and 3-months of storage.

1 month	ICC	Lower bound	Upper bound
Adiponectin	0.997	0.974	0.994
Ceruloplasmin	0.998	0.996	0.999
Hemopexin	0.994	0.987	0.997
MCP-1	0.998	0.996	0.999
3 month	**ICC**	**Lower bound**	**Upper bound**
Adiponectin	0.908	0.819	0.954
Ceruloplasmin	0.991	0.981	0.996
Hemopexin	0.949	0.898	0.975
MCP-1	0.998	0.996	0.999

Deming-Regression (Table 2) show that differences in means for each experimental condition are due to random error. Finally, Table 3 shows ICC for 1 month and 3 months storage conditions, revealing excellent reliability.

Discussion

In this study, we meaningfully add to the growing literature on using urinary biomarkers for LN evaluation. Exploration of stability is important in anticipation to move the RAIL into the clinical realm and consider use of the RAIL biomarkers in clinical trials.

We have previously shown the stability of NGAL and KIM-1 under different temperatures, freeze/thaw conditions and long-term storage (13). We now add four additional biomarkers of importance to lupus nephritis research: MCP-1, Adiponectin, Hemopexin and Ceruloplasmin. We tested effect of temperature, freeze/thaw, shipping, and storage for months, without significant differences between baseline and the experimental conditions. Importantly, we provide new data on the stability of urinary adiponectin, hemopexin, and ceruloplasmin that has not previously been reported.

We showed that the four other biomarkers included in the RAIL are stable at room temperature and 4 °C for up to 48 h. This has been replicated for other biomarkers as well. Wang et al. (18) evaluated effect of various temperature (4 °C, 22 °C, 40 °C) and short-term storage (24 h, 48 h) on the metabolite signature in urine. The control used was −20 °C. As in this study, they also found stability of the metabolite signature at 4 °C for 24 and 48 h, as well 22 °C for 24 h, however not 48 h. There was no integrity of the metabolite signature at 40 °C for any length of time. The effect of temperature on urinary KIM-1 and NGAL have been studied in various other studies (19, 20). Both proteins have been shown to be stable at 4 °C at 48 h as well as 25 °C at 48 h. NGAL has further been shown to be stable for up to 7 days at room temperature, 4 °C and −20 °C, which is not the case with KIM-1 (21, 22). Chang et al. (20), besides studying KIM-1 and NGAL, as well as other biomarkers, also evaluated MCP-1, and has shown it to be stable after 48 h at 4 °C, 48 h at 25 °C, no centrifugation but immediate aliquot and storage at −80 °C.

The data on longer-term storage are somewhat conflicting. Pennemans et al. (23) evaluated KIM-1 and showed good recovery at −80 °C for up to 1.5 years, but a bigger decline in biomarker concentration with storage at −20 °C. De Vrie et al. (22) evaluated KIM-1 and NGAL at −80 °C for 6 months, with both markers showing stability. Herrington et al. (24) showed urine albumin preservation for up to 6 months at −80 °C or −40 °C, but not −20 °C. In contrast, several authors have shown more variability in biomarker stability, even at −80 °C. Nauta et al. (25) evaluated multiple markers, including KIM-1, NGAL at 1 year in −20 °C or −80 °C. All markers except urine cystatin C showed a gradual decline in concentration, which seemed to stabilize after 6 months. Liu et al. (26) compared storage at −70 °C. KIM-1, NGAL, IL-18, liver-type fatty acid-binding protein (L-FABP) in hospitalized and non-hospitalized patients, where processing could be up to 6 h. Median duration of storage was 17.8 months for inpatient, and 14.6 months for outpatient samples. For NGAL, IL-18, L-FABP, storage time was not significantly associated with biomarker levels. KIM-1 levels were lower with longer storage times in the outpatient group, but this was finding was not found with the inpatient group.

The effect of freeze thaw cycles was studied for urine NGAL and KIM-1 previously. We newly evaluated the effects of freeze thaws on hemopexin, adiponectin, MCP-1 and ceruloplasmin concentrations. Hogan et al. (21) showed that NGAL was stable for up to 3 freeze/thaw cycles at −20 °C. Further, Herrington et al. (24) showed that urine albumin is preserved after 3 freeze/thaw cycles. These reports are similar to this study, with storage temperature of −80 °C. Pennemans et al. (23) performed a study in KIM-1, comparing the difference between 3 h and 24-h thaw period compared to 1 h, with optimal thawing time being 1 h.

Our study is the first to show the stability of biomarkers under study shipping conditions, both under dry ice and with wet ice. This has important implications for the measurement of these biomarkers by select clinical laboratories in stored urine samples from patients and from previous clinical trials.

One limitation with this study may be the small sample size, including of patients with lupus nephritis, which may reduce its generalizability. While most of the biomarkers were within the detectable range of the assay kits, ceruloplasmin had percentages of samples both above and below outside range of detection on assay. However, the amount below the detectable limit was reasonable, and the amount above detection that did not show large amount of decay.

In conclusion, our study demonstrates stability of hemopexin, ceruloplasmin, MCP-1 and adiponectin under different conditions that are important for clinical and experimental use, including up 48 h at room temperature, 4 °C,

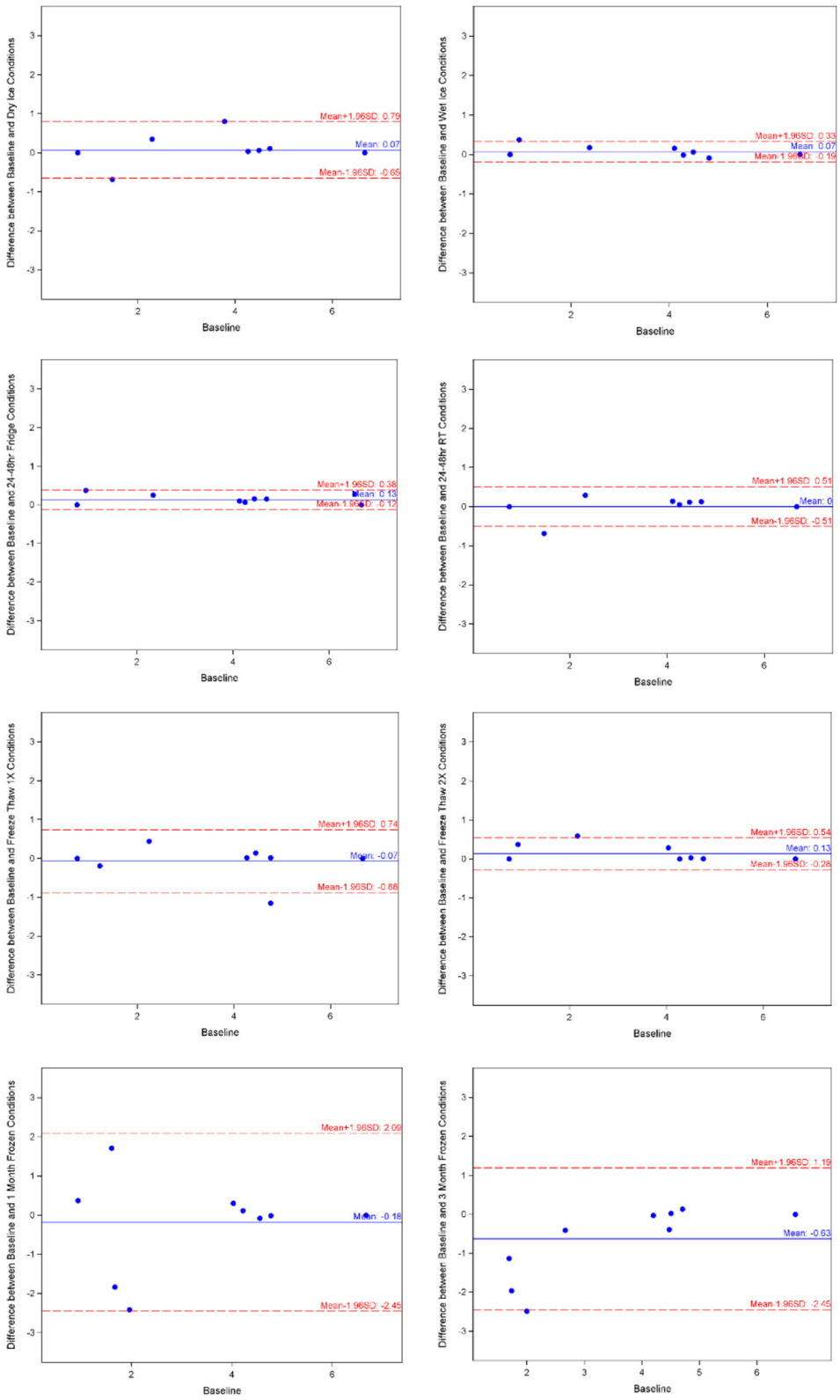

FIGURE 3
Ceruloplasmin Bland-Altman charts by experimental condition. Image made with BioRender.

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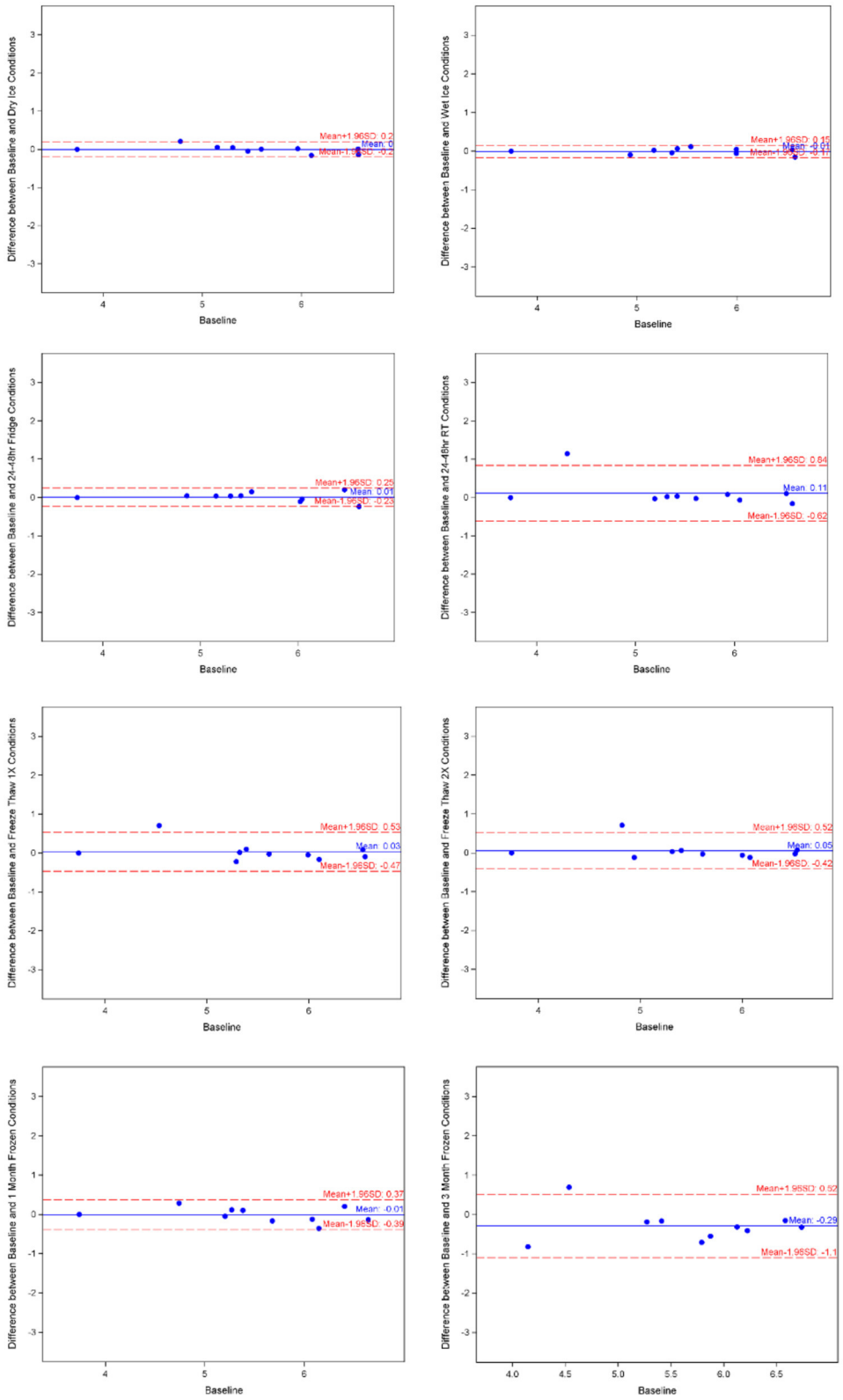

FIGURE 4
Hemopexin Bland-Altman charts by experimental condition. Image made with BioRender.

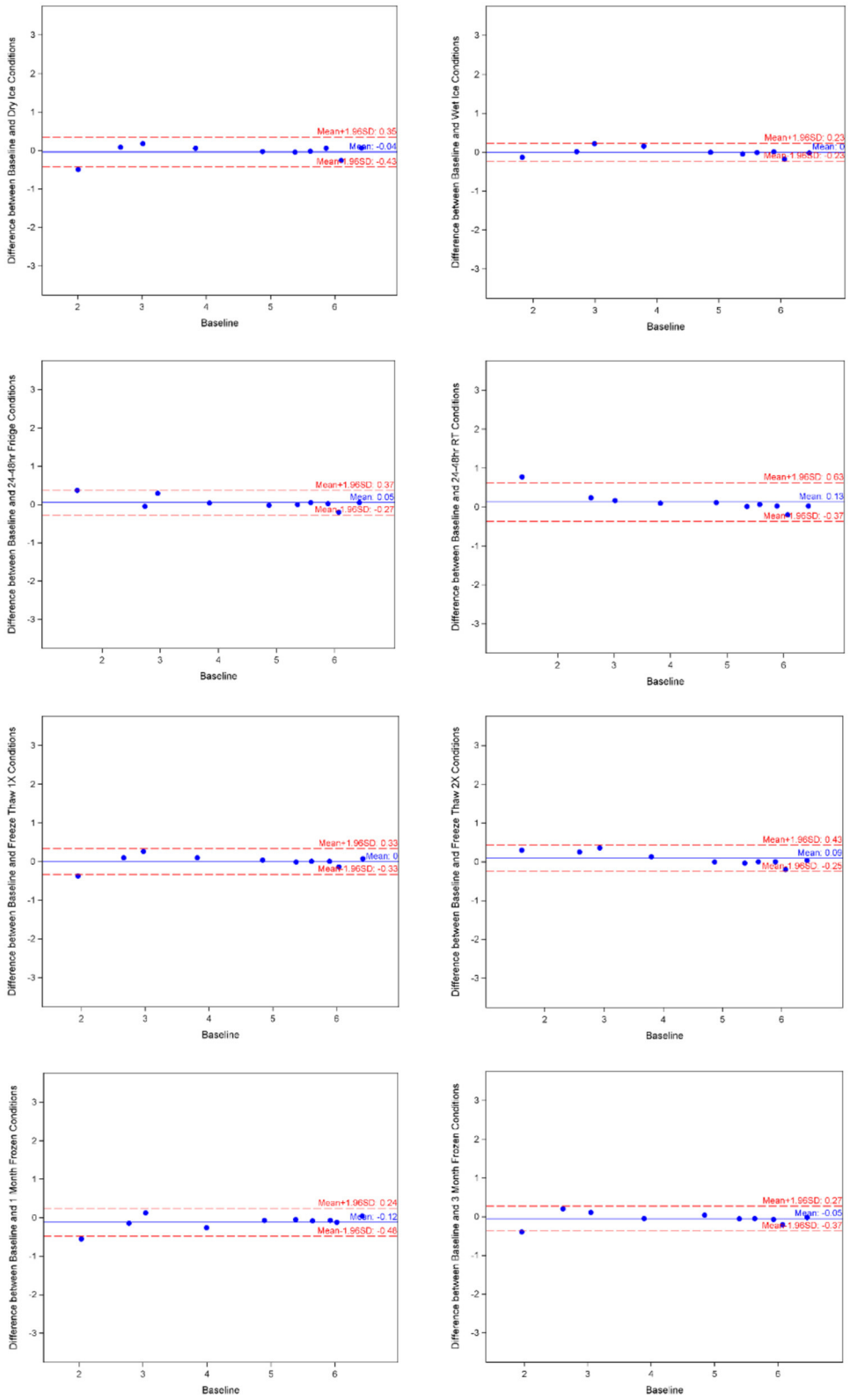

FIGURE 5
MCP-1 Bland-Altman charts by experimental condition. Image made with BioRender.

as well as up to 3 months at −80 °C storage. Importantly, we provide new data that these biomarkers are stable under storage with both wet and dry ice. With rising use of biomarkers for both clinical use and research, these results are reassuring that shipping, as well as long term storage and multiple freeze/thaw cycles can be employed prior to batch measurement.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by Cincinnati Children's Hospital IRB (IRB CCHMC-2008-0635). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

Research idea and study design: EC, HB, and PD. Data acquisition: JR. Data analysis and interpretation: BH, EC, HB, and PD. Statistical analysis: BH and TQ. Supervision or mentorship: HB and PD. All authors contributed to the article and approved the submitted version.

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Conflict of interest

Author PD is a co-inventor on submitted patents for the use of NGAL as a biomarker of kidney disease.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Case report: A pediatric case of MPO-ANCA-associated granulomatosis with polyangiitis superimposed on post-streptococcal acute glomerulonephritis

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An eight-year-old girl was admitted with vomiting, gross hematuria, and progressive renal dysfunction. A renal biopsy revealed endocapillary proliferative glomerulopathy and crescent formation. Immunofluorescence staining revealed diffuse granular deposits of IgG and C3. Post-streptococcal acute glomerulonephritis (PSAGN) was suspected, based on the elevated anti-streptolysin O levels, decreased serum C3 concentrations, and histologic findings. The myeloperoxidase anti-neutrophil cytoplasmic antibody (MPO-ANCA) test was positive, and the young patient gradually developed palisaded neutrophilic and granulomatous dermatitis (PNGD), orbital and paranasal sinus granulomatous neoplasms, along with intermittent nose, head, and orbital pain. Finally, she was diagnosed with the rare MPO-ANCA-associated granulomatosis with polyangiitis (GPA) superimposed on PSAGN. The patient was treated with aggressive renal replacement therapy, methylprednisolone pulse therapy, and intravenous pulse cyclophosphamide; her renal function normalized, and her pain symptoms improved.

KEYWORDS

ANCA-associated vasculitides, post-streptococcal acute glomerulonephritis, granulomatosis with polyangiitis, MPO-ANCA, immunosuppressive therapy, immunosuppressive therapy

Introduction

Antineutrophil cytoplasmic antibody-associated vasculitides (AAVs) are a group of life-threatening primary systemic necrotizing small-vessel vasculitides characterized by the presence of antineutrophil cytoplasmic antibodies (ANCA), including granulomatosis with polyangiitis (GPA), microscopic polyangiitis (MPA), and eosinophilic granulomatosis with polyangiitis (EGPA), classified by clinical features. GPA and MPA frequently affect older adults, while EGPA tends to affect young adults. Approximately 60% of AAV cases involve the kidney; this condition is called ANCA-associated glomerulonephritis (AAGN), which can be diagnosed by a renal biopsy and typically manifests as necrotizing and crescentic glomerulonephritis with little to no immunoglobulin (Ig) or complement deposition. Cases progress to end-stage renal disease (ESRD) in 20%–25% of patients, and the renal prognosis of AAV is usually very poor.

Post-streptococcal acute glomerulonephritis (PSAGN) is an infection-related glomerulonephritis (IRGN) caused by group A β-hemolytic streptococci and characterized

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by endocapillary hypercellularity with immunoglobulin G (IgG) and complement (C3) deposition. Renal damage in PSAGN is caused by the activation of the classical and alternative complement pathways secondary to streptococcal-initiated humoral immunity. In the early stages of PSAGN, serum C3 levels are low and gradually return to the normal range within two months. PSAGN usually has a favorable renal prognosis and requires only suggestive therapy.

In this report, we describe a pediatric case of PSAGN, as suggested by laboratory test results and a renal biopsy, with progressive renal dysfunction that was alleviated by hemodialysis and immunosuppressive treatment. At baseline, MPO-ANCA was detected; during the course of the disease, granulomatous dermatitis and orbital and paranasal sinus granulomas gradually appeared. One case of AAV superimposed on a PSAGN presented with more severe renal dysfunction; however, the exact etiology of this condition remains unknown.

Case description

An eight-year-old Chinese girl was admitted with complaints of vomiting, oliguria (150–300 ml/day), and gross hematuria for two days. She had no symptoms of fever, skin infection, or joint pain. Her blood pressure was elevated (122/80 mmHg). Serum laboratory tests revealed mild anemia (hemoglobin 96 g/L), severe acute kidney injury (blood urea nitrogen 35.1 mmol/L, creatinine 564.9 μmol/L, uric acid 610 μmol/L), and abnormal urine tests (185.6 RBCs/HPF, urine protein 0.63 g/24 h). Serum C3 concentration was 0.051 g/L (normal range: 0.9–1.8 g/L), anti-streptolysin O(ASO) was 682 IU/ml, and erythrocyte sedimentation rate (ESR, 54 mm/h) was elevated. Anti-nuclear antibody (1:100), anti-histone antibody+, MPO-ANCA 38.88R U/ml (by ELISA, normal range:<20 RU/ml), p-ANCA (+). Tests for other laboratory indicators, including procalcitonin, C-reactive protein (CRP), tumor markers, anti-cardiolipin antibody, and anti-glomerular basement antibody were

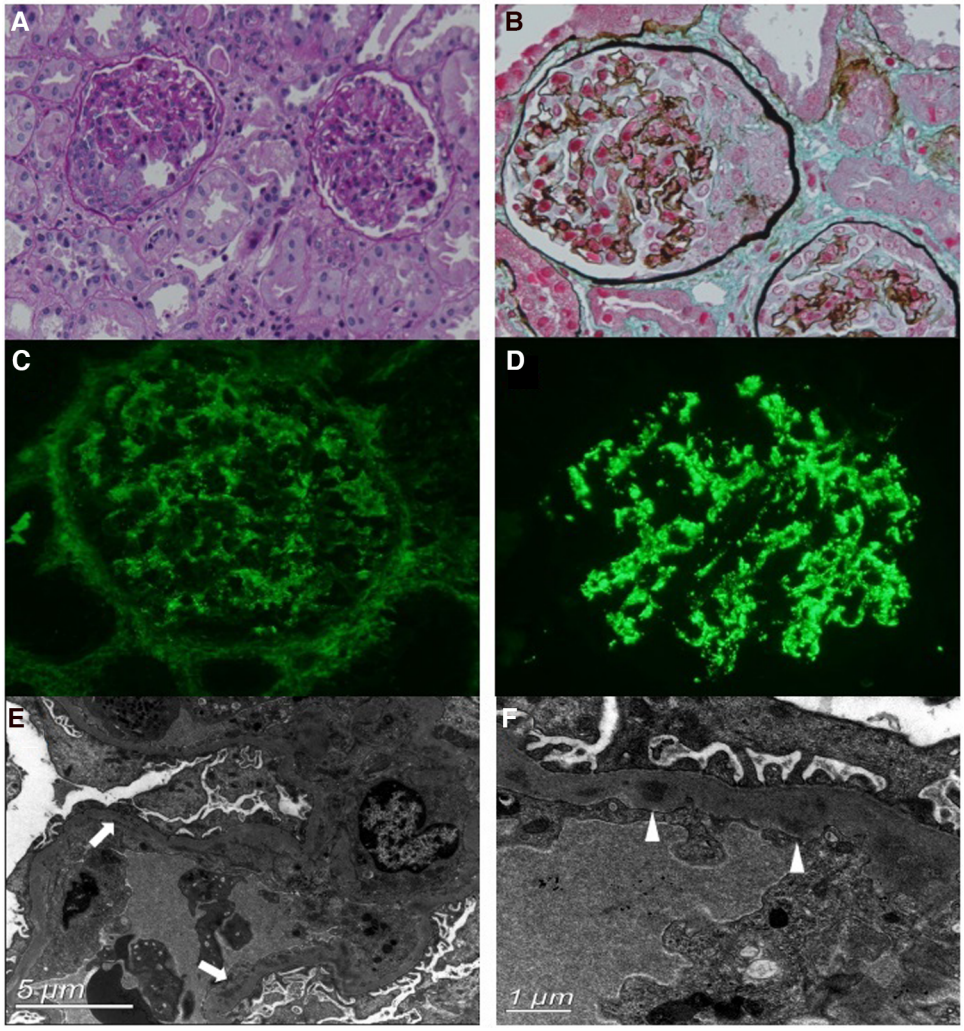

FIGURE 1
Renal biopsy findings. (A,B) Light microscopy (PAS and Masson's stain, ×200 magnification) shows the cellular crescent formation and diffuse inflammatory cell infiltration. (C,D) Immunofluorescence staining shows diffuse, granular deposits of IgG (C) and C3 (D) along the mesangial region and glomerular capillary walls. (E,F) Electron microscopy reveals electron-dense deposits in the segmental subendothelial region (arrows) and glomerular basement membrane (GBM) (triangles).

negative. CT scans of the chest and abdomen were normal. Renal ultrasonography showed that the size of the right kidney was 115 × 47 mm^2, the left kidney was 114 × 46 mm^2, and the echo of the renal cortex was enhanced.

A renal biopsy was performed. Light microscopy revealed that among the 22 glomeruli, eight had cellular crescent formation and one had segmental sclerosis (**Figures 1A,B**). Furthermore, diffuse inflammatory cell infiltration, predominantly neutrophils and macrophages, was observed in the glomerular capillary lumen. The capillary wall appeared to be thickened. The tubulointerstitium displayed moderate acute injury, and the focal brush border of the tubular epithelium was detached. Immunofluorescence staining revealed diffuse granular deposits of IgG and C3 (IgG +, C3 +++) along the mesangial region and glomerular capillary walls (**Figures 1C,D**). Electron microscopy revealed electron-dense deposits in the mesangium and glomerular basement membrane (GBM) and intermittently in the sub-endothelial region (**Figures 1E,F**). No sub-epithelial electron-dense deposits were observed. In short, the renal pathology revealed endocapillary proliferative glomerulopathy. Complement component analysis and whole-exon sequence gene detection were normal.

Based on the clinical and histologic findings, the patient was diagnosed with PSAGN. However, it seemed to be an atypical case of PSAGN. Given the young patient's rapidly progressive clinical course and extensive crescent formation confirmed by renal biopsy, she was administered two pulses of methylprednisolone (500 mg/day, 20 mg/kg), followed by 40 mg (1.6 mg/kg) of oral prednisone per day, and hemodialysis was performed twice. After 20 days of treatment, serum creatinine levels returned to 29.9 μmol/L, hemoglobin increased to 114 g/L, and urine protein also decreased to 0.1 g/24 h. The serum C3 level was 1.3 g/L. Blood pressure (98/62 mmHg) and increased kidney size (96 × 37 mm^2, 100 × 41 mm^2) also went back to normal. Considering that the laboratory indicators returned to normal, other immunosuppressive agents were not added temporarily, and oral prednisone was tapered (**Figure 2**).

Approximately five months later, the patient developed recurrent systemic red rashes (**Figures 3A,B**). Laboratory tests revealed normal urine tests and renal function, p-ANCA+, MPO-ANCA 71.58 RU/ml, and a skin biopsy showed palisaded neutrophilic and granulomatous dermatitis (PNGD). Therefore, prednisone was added at 40 mg/day, and mycophenolate mofetil (20 mg/kg) was prescribed to control ANCA activation. The rash gradually resolved (**Figure 2**).

One and a half years later, the patient presented with a convex left eye and intermittent nose, head, and orbital pain with normal hearing. The bridge of the nose was low and flat, resembling a saddle nose (**Figures 3C,D**). Imaging revealed orbital pseudotumors and bilateral sinusitis. The patient underwent eye surgery, and the pathology of the left ocular mass indicated chronic inflammation with granulomas and necrosis. We simultaneously prescribed prednisone (15 mg/day), a single dose of rituximab (0.4 g, 375 mg/m^2), and suspended mycophenolate mofetil. However, the patient's symptoms of nose, head, and orbital pain did not improve, ESR and CRP were elevated (ESR 38 mm/h, CRP 19.6 mg/L), p-ANCA was still positive, and MPO-ANCA was 193 RU/ml. A sinus biopsy

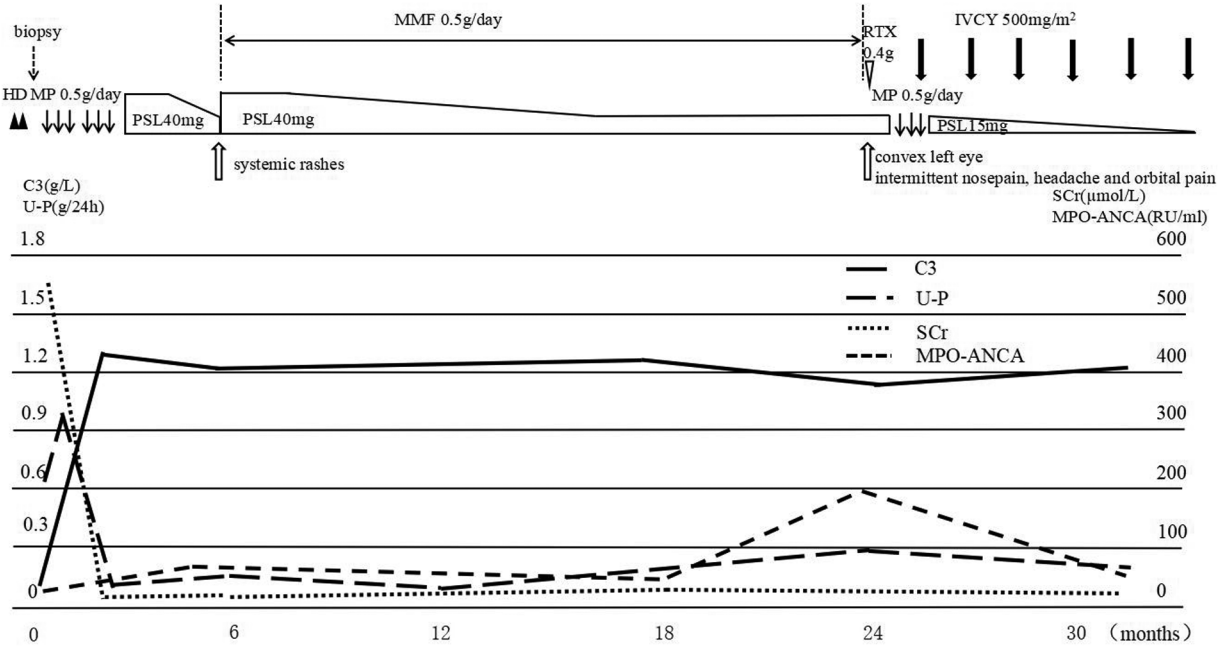

FIGURE 2
The clinical course of the patient. After hemodialysis and two pulses of methylprednisolone (500 mg/day), followed by 40 mg oral prednisone per day, serum C3 and SCr levels returned to normal, while MPO-ANCA was still positive. Subsequently, the patient developed systemic rashes; prednisone was increased to 40 mg/day, and mycophenolate was prescribed. Two years later, the girl presented with orbital and paranasal sinus abnormalities, the MPO-ANCA level was highly elevated, treatment with one dose of rituximab was unsuccessful, and another pulse of methylprednisolone combined with monthly IVCY brought the disease under control. MP, methylprednisolone pulse; PSL, prednisolone; IVCY, intravenous cyclophosphamide; U-P, urine protein.

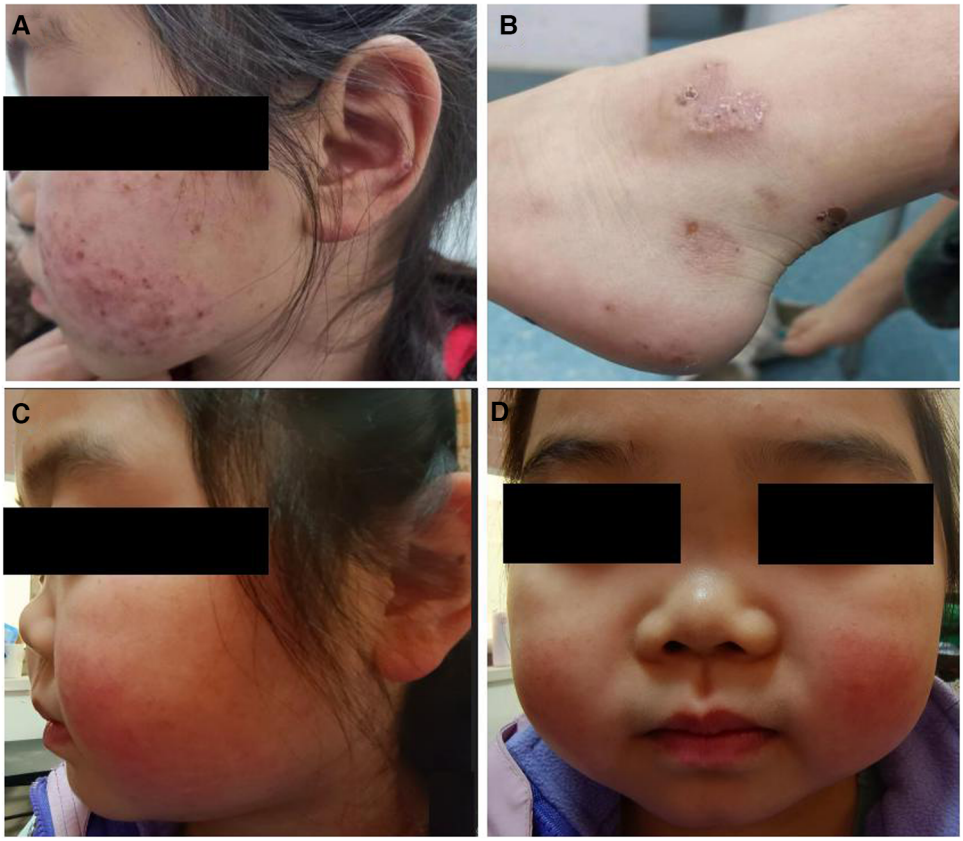

FIGURE 3
Clinical characteristics of the patient. (A,B) Systemic red rash. (C,D) Cartilage involvement, showing saddle nose deformity.

revealed numerous granulomatous neoplasms in both maxillary sinuses. Consequently, the patient was administered one pulse of methylprednisolone (500 mg/day) for three days, followed by oral prednisone at 15 mg/day. Pulsed cyclophosphamide was then administered at 500 mg/m^2 per dose for six doses every month. During treatment, the patient's urine and renal function remained normal, and there were no apparent lung lesions. The pain in the nose, eyes, and head gradually subsided.

Discussion and conclusions

ANCA is usually thought to be the pathogen in AAV, which is an autoantibody that is directly against the cytoplasm of neutrophils and monocytes and includes mainly cytoplasmic (C-ANCA) and perinuclear (P-ANCA) forms. ANCA is specific to the diagnosis of GPA and MPA. C-ANCA occurs in 95% of new-onset GPA cases, P-ANCA in 80% of new-onset MPA cases, and 40% of new-onset EGPA cases. The prevalence of AAV is approximately 200–400 per million people, and it is rare in children (1). In children, AAV has a higher female preponderance. The peak incidence of AAV occurs in the second decade of life; the median age at diagnosis is 12–14 years; and GPA is more common than MPA or EGPA (2). Clinically, the prognosis of AAV is poor, and immunosuppressive therapy is warranted.

In our case, the clinical course of rapidly progressive abnormal renal function, positive MPO-ANCA at disease onset, and massive crescent formation proven by renal biopsy suggested the possibility of an AAV diagnosis. Subsequently, the girl developed PNGD, orbital and paranasal sinus granulomatous neoplasms, and a saddle nose deformity, accompanied by persistently positive MPO and P-ANCA according to the American College of Rheumatology/European Alliance of Associations (ACR/EULAR) (3) classification criteria, and was diagnosed with GPA. GPA was first described in 1937. The main clinical manifestations of GPA are granulomatous inflammation (orbital pseudotumor, chronic sinusitis, Eustachian tube dysfunction, etc.) and small- or medium-vessel vasculitis (pulmonary hemorrhage, glomerulonephritis, skin purpura, etc.). Upper airway abnormalities, manifesting as chronic rhinitis and serous otitis, may be the earliest presenting features and are present in more than 90% of cases, while abnormalities in the kidneys and lungs are present in 80% and 85% of cases, respectively (4). Historically, GPA has been diagnosed based on the presence of a histopathologic triad including necrotizing angiitis, granulomatous inflammation, and necrotizing crescentic glomerulonephritis (5). The incidence of GPA in the Asian population ranges from 0.37–2.1 per million people per year, and its prevalence in China is approximately 0.194 per million people. Most GPA patients in India and Korea are PR3 positive, while 60% of the GPA patients in China are MPO-ANCA positive (6). In addition, it has been

confirmed that the ANCA serotype is more predictive of disease regression and clinical outcomes (7–9). A cohort analysis enrolled 365 patients diagnosed with AAV, 44 (12%) with MPA, and 321 (88%) with GPA. Among the 321 patients with GPA, 273 (85%) had PR3-ANCA, 33 (10%) had MPO-ANCA, and 15 (5%) remained ANCA negative. Compared with MPO-ANCA-positive MPA patients, MPO-ANCA-positive GPA patients were younger at diagnosis, and MPO-ANCA-positive GPA was predominantly female (10). Another case-control study showed that patients with MPO-ANCA-positive GPA were less likely to have severe disease (11) and had lower mortality and higher relapse rates (12). Therefore, it seems that some differences exist between MPO-ANCA-positive and PR3-ANCA-positive patients with GPA in terms of clinical manifestations, they may be different diseases (13).

Finally, the patient was diagnosed with an MPO-ANCA-associated GPA. Although recurrent episodes occurred, no fatal alveolar hemorrhage developed, and renal function quickly returned to normal. These clinical features are consistent with the above observations.

The pathogenesis of AAV is still poorly understood. Two familiar hypotheses are the complementary peptide model and the molecular mimicry model. However, evidence has shown that other immune cell mediators, such as CD4+ T cells, may be involved in AAV onset. Complement activation has been associated with the pathogenesis and progression of AAV. Some studies have reported immunocomplex (IC) formation during the early phases of AAV (14, 15). IC deposition is common in patients with AAGN, and C3 deposition is found in 30%–40% of patients with AAGN and is also an independent risk factor for AAV prognosis (16, 17). The renal biopsy of this patient revealed diffuse granular deposits of IgG and C3 (IgG +, C3 +++), which were incompatible with pauci-immune glomerulonephritis and showed more severe renal involvement in the early stage of the disease course, requiring renal replacement therapy.

ANCA can be caused by environmental exposures (silica), drug use (hydralazine, propylthiouracil, penicillamine, etc.), and disease (chronic inflammatory diseases, neoplasms, and infections). ANCA can also be caused by infection, and the possible mechanisms linking infection and ANCA include the production of neutrophil extracellular traps (NETs) and the ligation of toll-like receptors (TLRs) (18). Infection is associated with the morbidity of various glomerular diseases; the recently proposed concept of streptococcal infection-related nephritis (SIRN) includes IgAN, AAV, PSAGN, and so on. Streptococcal infection and ANCA act synergistically or coincidentally; however, the exact mechanism underlying the streptococcal infection associated with AAV remains unclear. In general, PSAGN patients had a good prognosis in the absence of immunosuppressive therapy. Crescent formation is not rare in PSAGN and are a predictor of poor long-term prognosis (19). In New Zealand children, 41% (11 of 27) of patients with crescentic PSAGN had higher serum creatinine levels, requiring acute dialysis (20). In addition, PSAGN can be associated with many other diseases, such as atypical hemolytic uremic syndrome (aHUS) (21), arthritis (19), IgAN (22), membranous nephropathy (23), and Alport syndrome (22). The co-occurrence of PSAGN and AAV is relatively rare (24, 25). The critical task is to decide whether additional treatment of the ANCA-associated disease is needed or only treatment of the infection. Ardiles et al. (26) tested serum IgG-ANCA levels in 210 patients with PSAGN and 14 patients with streptococcal impetigo without glomerular disease. In the PSAGN group, ANCA was detected in 9% of the patients. However, none of the subjects with streptococcal impetigo tested positive in this study. ANCA was associated with higher serum creatinine levels and more crescent formation, suggesting that ANCA may play a pathogenic role in kidney disease. Therefore, we prescribed glucocorticoids, and the renal function and urine abnormalities recovered quickly. When orbital and paranasal sinus granulomatous neoplasms developed, the combination of prednisone and pulsed cyclophosphamide controlled the disease.

In conclusion, we reported a rare case of MPO-ANCA-associated GPA superimposed on post-streptococcal acute glomerulonephritis, in which the prognosis was relatively good after active hemodialysis and immunosuppressive treatment. In cases of PSAGN with ANCA, although the initial ANCA titer is not very high, we recommend referring to the treatment principle of AAV, and aggressive and long-term maintenance of immunosuppressive therapies is required. A closer follow-up is warranted to focus on extrarenal manifestations and ANCA levels. Given the small number of cases of PSAGN with AAV, the pathophysiologic features and long-term renal prognosis remain unclear.

Data availability statement

The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding authors.

Ethics statement

Written informed consent was obtained from the minor(s)' legal guardian/next of kin for the publication of any potentially identifiable images or data included in this article.

Author contributions

QK and XH: collecting history, manuscript editing. LJ, ZZ, and CGu: literature search. CGa and ZX: manuscript review. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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The changes of Treg and Th17 cells relate to serum 25(OH)D in patients with initial-onset childhood systemic lupus erythematosus

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Background: T helper 17 (Th17) cells and regulatory T cells (Treg) are known to play a crucial role in the pathogenesis of systemic lupus erythematosus (SLE). Improving the balance between Treg and Th17 cells can be a promising new therapeutic target in SLE patients. Vitamin D has a significant impact on the immune inflammatory process and the immune cells involved in this process. The purpose of this study is to investigate the relationship between Th17, Treg, cytokines, and serum 25 hydroxyvitamin D [25(OH)D] in patients with initial-onset childhood SLE.

Methods: A total of 82 children aged <18 years with initial-onset SLE were included, as well as 60 healthy subjects during the same period at the Pediatrics Department of the Second Hospital of Hebei Medical University. The chemiluminescence method was performed to detect serum 25(OH)D levels. Flow cytometry was used to evaluate Treg and Th17 cells. An enzyme-linked immunosorbent assay kit was used to evaluate plasma interleukin (IL)-23, IL-17, IL-10, IL-6, and tumor necrosis factor alpha (TNF-α) concentrations.

Result: The serum 25(OH)D levels in patients with initial-onset childhood SLE were significantly lower than those in the healthy controls. The proportion of lupus nephritis (LN) was higher in the vitamin D insufficiency group (71.4%) compared with the vitamin D sufficiency group (30.3%) ($p < 0.05$). The SLE disease activity index (SLEDAI) was higher in the vitamin D insufficiency group (median = 14) than that in the vitamin D sufficiency group (median = 9) ($p < 0.05$).The 25(OH)D level was positively correlated with the Treg ratio ($r = 0.337$, $p = 0.002$), and it was negatively correlated with the Th17 cell ratio ($r = -0.370$, $p = 0.001$). The serum 25(OH)D level had a negative correlation with IL-23 ($r = -0.589$, $p < 0.001$), IL-17($r = -0.351$, $p = 0.001$), TNF-α ($r = -0.283$, $p = 0.01$), IL-6 ($r = -0.392$, $p < 0.001$), and IL-10 ($r = -0.313$, $p = 0.004$) levels.

Conclusion: The serum 25(OH)D levels decreased in patients with initial-onset childhood SLE. There was a negative correlation between the serum 25(OH)D levels and SLEDAI. The serum 25(OH)D levels in patients with initial-onset childhood SLE were negatively correlated with the Th17 ratio and related cytokines, while positively correlated with the Treg ratio.

KEYWORDS

25(OH)D, SLE, Treg, Th17, cytokines

1. Introduction

Systemic lupus erythematosus (SLE) is a chronic systemic autoimmune disease that causes chronic inflammation and damages multiple tissues and organs, including the central nervous system, skin mucosa, cardiovascular system, kidneys, and joints. The pathogenesis of SLE is not fully understood. SLE is characterized by polyclonal activation of T and B lymphocytes. In addition to an imbalance of T helper 1 (Th1) and T helper 2 (Th2) cells, the regulatory T cells (Treg) and T helper 17 (Th17) cells are known to play a crucial role in the pathogenesis of SLE (1). Studies have found that quantity anomalies or/and functional defects of Treg and Th17 cells were associated with flares and organ damages in SLE patients (2). Th17 cells secrete a profile of potent pro-inflammatory cytokines, including interleukin-17 (IL-17), and potent tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6) upon certain stimulation (3). Interleukin-23 (IL-23) is an important cytokine that promotes the secretion of interleukin-17 by Th17 cells to maintain pathological status by combining with IL-23 receptor (4). Although it is widely believed that Treg cells play a preventive role in autoimmunity, the data on SLE are inconsistent (5). Interleukin-10 (IL-10) is secreted not only by Th2 cells but also by Treg cells. The differentiation and proliferation of Treg and Th17 cells are regulated by multiple cytokines including IL-10, IL-23, IL-17, and IL-6 (6). Regulating the balance between Treg and Th17 cells will be a promising new therapeutic target in SLE patients.

Vitamin D is an important steroid hormone that has significant effects on bone health and the cardiovascular system (7). Vitamin D also has some non-classical effects, such as immune modulatory effects (8). Many studies have found that most patients with autoimmune diseases worldwide suffer from vitamin D deficiency. These studies have also emphasized the relationship between decreased serum vitamin D levels and disease activity in SLE and rheumatoid arthritis (9–11). Vitamin D has great impact on immune cells as well as the inflammatory cascade. The receptors of Vitamin D are commonly accessible for many adaptive immune cells including T cells, B cells, macrophages, and dendritic cells (12).

Whether vitamin D can act on Treg and Th17 cells remains largely unexplored. Therefore, the purpose of this study is to investigate the relationship between Th17, Treg, cytokines, and serum 25 hydroxyvitamin D [25(OH)D] in patients with initial-onset childhood SLE.

2. Patients and methods

2.1. Study subjects

A total of 82 children aged <18 years with initial-onset SLE who were admitted to the Pediatrics Department of the Second Hospital of Hebei Medical University between April 2020 and February 2023 were included in this study. All patients met the 1997 American College of Rheumatology (ACR) classification criteria for SLE (13) or the 2012 Systemic Lupus Erythematosus International Collaborating Clinics (SLICC) classification criteria for SLE (14). The disease activity was assessed using the SLE Disease Activity Index-2000 (SLEDAI-2K). The exclusion criteria were as follows: certain diseases that affect vitamin D metabolism (gastrointestinal surgery, liver metabolic diseases, tumors, etc.); and vitamin D supplementation by oral medication within the past 3 months. This study involved 60 healthy subjects during the same period as healthy controls (HC). This study was approved by the ethics committee of the Second Hospital of Hebei Medical University (protocol number 2021-R307).

2.2. Laboratory examinations

Laboratory examinations included routine blood tests, 24-h urine protein, erythrocyte sedimentation rate (ESR), liver function, renal function, complement 3 (C3), complement 4 (C4), antinuclear antibody, double-stranded deoxyribonucleic acid (dsDNA), serum calcium, and serum phosphorus.

2.3. Determination of serum 25(OH)D level

Blood was collected between 6:00 and 7:00 in the morning, and the children were fasted from food and water overnight before the blood samples were collected. The chemiluminescence method was performed for the detection of serum 25(OH)D levels, the kit was provided by Siemens Healthcare Diagnostics Inc. (USA), and the analysis was done using an ADVIA Centaur XP automatic chemiluminescence immunoassay analyzer. A vitamin D insufficiency was defined as serum 25(OH)D level of < 20 ng/ml, and a vitamin D sufficiency was defined as serum 25(OH)D level of ≥ 20 ng/ml.

2.4. Flow cytometry

2.4.1. Sample and cell preparations

All participants fasted from water after 12 p.m. the previous day, and peripheral venous blood samples of approximately 5 ml were collected between 6:00 and 7:00 in the morning. Blood samples were anticoagulated with ethylenediaminetetraacetic acid dipotassium (EDTA-K2), which was used to isolate and identify Treg and Th17 cell subsets. Peripheral blood mononuclear cells (PBMCs) were obtained through Ficoll density gradient. PBMCs were suspended at a density of 2×10^6 cells/ml on a complete culture medium (RPMI 1640 supplemented with 100 μg/ml streptomycin, 100 U/ml penicillin, 2 mM glutamine, and 10% heat-inactivated fetal calf serum) to obtain and analyze Th17 cell subset. The cell suspension was transferred to 24-well culture plates with a concentration of 25 ng/ml of phorbol ethyl ester (PMA), 1.7 ml of moneomycin (MN), and 1 ml of ionomycin (LC), and then incubated at 37°C under a 5% CO_2 environment for 4 h. For Treg cells analysis, PBMCs were suspended at a density of 2×10^7 cells/ml.

2.4.2. Surface and intracellular staining

To analyze Th17 cell subset, the cells were fixed and permeabilized according to the manufacturer's instructions, and then intracellularly stained with PE-conjugated anti-IL-17 monoclonal antibodies. Th17 cells were labeled as CD4 + IL-17A+. For Treg analysis, the cells were surface-stained, and then fixed permeabilized, then stained with PE anti-human Foxp3 according to the manufacturer's instructions. Treg cells were labeled as CD4 + CD25 + FoxP3+. Homotypic controls were used to verify specificity and perform compensation correction. All antibodies were provided by eBioscience. Stained cells were analyzed by flow cytometry analysis using a FACSCalibur flow cytometer (BD biosciences) with FlowJo software (Tree Star, San Carlos, CA, USA).

2.4.3. Enzyme-linked immunosorbent assay

A total of 3 ml of blood was collected and anticoagulated with EDTA-K2 to evaluate cytokines. Plasma IL-23, IL-17, TNF-α, IL-6, and IL-10 concentrations were determined by using human IL-23, IL-17, TNF-α, IL-6, and IL-10 enzyme-linked immunosorbent assay (ELISA) kit (Elabscience, Elabscience Biotechnology Co., Ltd.).

2.5. Statistical analysis

The data were statistically analyzed by using the SPSS version23.0 program. The data were presented as the mean ± standard deviation (SD) or median, and the categorical variables were expressed as frequencies and percentages. The rates were compared between two or more groups using chi-square test or Fisher's exact test. A non-parametric Mann–Whitney *U* test was used to compare the data between groups. Pearson correlation analysis was used for variables that conformed to a normal distribution, and Spearman correlation analysis was used for variables that did not conform to a normal distribution. Statistically significant was defined as *p*-value less than 0.05.

3. Result

3.1. Serum 25(OH)D level

As shown in **Figure 1**, the serum 25(OH)D levels in patients with initial-onset childhood SLE were significantly lower than those in the healthy control group (25.60 ± 6.87 ng/ml for HC, 18.86 ± 5.18 ng/ml for SLE).

3.2. Comparison of clinical and laboratory findings in pediatric SLE patients with different 25(OH)D levels

In this study, the patients with initial-onset childhood SLE were divided into two groups based on their serum 25(OH)D levels. The clinical manifestations and laboratory parameters were compared between the two groups. The proportion of lupus nephritis (LN) was higher in the vitamin D insufficiency group (71.4%) compared with the vitamin D sufficiency group (30.3%) ($p < 0.05$). SLEDAI was higher in the vitamin D insufficiency group (median = 14) than that in the vitamin D sufficiency group (median = 9) ($p < 0.05$). The probability of pulmonary involvement and anemia was higher in the vitamin D insufficiency group. The SLEDAI-2K score was higher in the vitamin D insufficiency group (median = 14) than that in the vitamin D sufficiency group (median = 9) ($p < 0.05$), indicating higher disease activity in SLE. Compared with the vitamin D sufficiency group (0.54 ± .024 g/L), SLE patients in the vitamin D insufficiency group had lower levels of C3 (0.39 ± 0.27 g/L) ($p < 0.05$). It was worth noting that SLE children with insufficient vitamin D had lower serum calcium levels (**Table 1**).

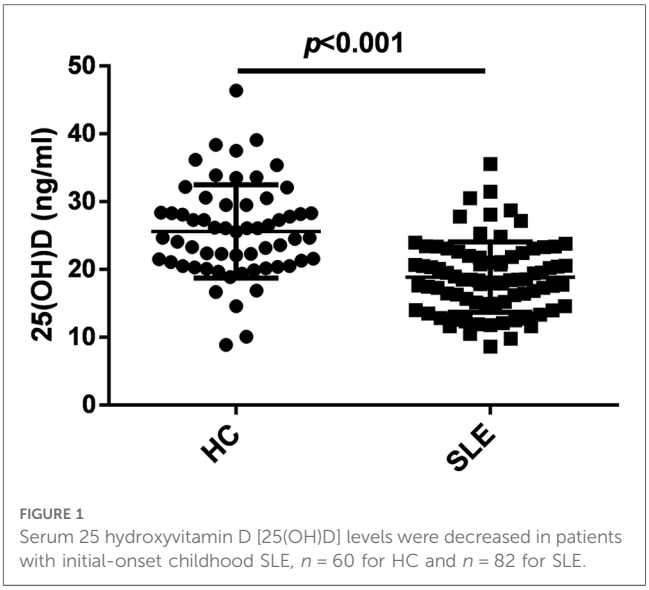

FIGURE 1
Serum 25 hydroxyvitamin D [25(OH)D] levels were decreased in patients with initial-onset childhood SLE, *n* = 60 for HC and *n* = 82 for SLE.

3.3. Correlations of 25(OH)D levels with the clinical and laboratory parameters

The 25(OH)D levels were positively correlated with C3 ($r = 0.303$, $p = 0.006$) and C4 ($r = 0.225$, $p = 0.042$), while the 25(OH)D levels were negatively correlated with 24-h urinary protein ($r = -0.423$, $p < 0.001$) (**Table 2**).

3.4. Correlations of the Treg ratio with the clinical and laboratory parameters

The Treg ratio was positively correlated with C4 ($r = 0.281$, $p = 0.011$), while it was negatively correlated with 24-h urinary protein ($r = -0.261$, $p = 0.018$) and SLEDAI ($r = -0.268$, $p = 0.015$) (**Table 3**).

3.5. Correlations of the Th17 cell ratio with the clinical and laboratory parameters

The Th17 cell ratio was positively correlated with 24-h urinary protein ($r = 0.277$, $p = 0.012$) and SLEDAI ($r = 0.287$, $p = 0.009$),

TABLE 1 Demographic, clinical, and laboratory characteristics of the studied groups.

Characteristic	Total	25(OH)D < 20 ng/ml	25(OH)D ≥ 20 ng/ml	*p*
Female sex, n/N (%)	67/82 (81.7%)	40/49 (81.6%)	27/33 (81.8%)	0.983
Age (years), mean ± SD	11.4 ± 2.2	11.6 ± 2.4	11.2 ± 2.0	0.320
Lupus nephritis, *n* (%)	45 (54.9%)	35 (71.4%)	10 (30.3%)	**<0.001**
Arthritis, *n* (%)	22 (26.8%)	14 (28.6%)	8 (24.2%)	0.664
Mucocutaneous, *n* (%)	39 (47.6%)	23 (46.9%)	16 (48.5%)	0.891
Vasculitis, *n* (%)	1 (1.2%)	1 (2%)	0 (0%)	0.598
Serositis, *n* (%)	8 (9.8%)	7 (14.3%)	1 (3%)	0.092
Neurologic, *n* (%)	16 (19.5%)	9 (18.4%)	7 (21.2%)	0.750
Pulmonary, *n* (%)	13 (15.9%)	11 (22.4%)	2 (6.1%)	**0.046**
SLEDAI, median (range)	12 (2–33)	14 (2–33)	9 (3–31)	**0.048**
Leukopenia (<4 × 10^9/L), *n* (%)	42 (51.2%)	21 (42.9%)	21 (63.6%)	0.065
Anemia (<110 g/L), *n* (%)	46 (56.1%)	33 (67.3%)	13 (39.4%)	**0.012**
Thrombocytopenia (<100 × 10^9/L), *n* (%)	27 (32.9%)	20 (40.8%)	7 (21.2%)	0.064
Proteinuria, *n* (%)	43 (52.4%)	35 (71.4%)	8 (24.2%)	<0.001
C3 (g/L), mean ± SD	0.45 ± 0.27	0.39 ± 0.27	0.54 ± 0.24	**0.004**
C4 (g/L), mean ± SD	0.07 ± 0.06	0.07 ± 0.06	0.08 ± 0.05	0.055
Positive anti-dsDNA, *n* (%)	52 (63.4%)	33 (67.3%)	19 (57.6%)	0.368
Serum calcium (mmol/L), mean ± SD	2.17 ± 0.16	2.11 ± 0.16	2.26 ± 0.12	**<0.001**
Serum phosphorus (mmol/L), mean ± SD	1.55 ± 0.43	1.61 ± 0.47	1.46 ± 0.33	0.356

Statistically significant results are highlighted in bold.

while it was negatively correlated with C3 ($r = -0.257$, $p = 0.02$) (Table 4).

3.6. The relationship between 25(OH)D levels and the proportion of Treg and Th17 cells

As shown in Figure 2A, the Treg ratio in children with initial-onset childhood SLE decreased (5.69 ± 2.03 for HC, 2.79 ± 1.33 for SLE). The 25(OH)D levels were positively correlated with the Treg ratio ($r = 0.337$, $p = 0.002$) (Figure 2B). As shown in Figure 2C, the Th17 cell ratio in patients with initial-onset childhood SLE increased (3.52 ± 1.36 for HC, 8.16 ± 6.16 for SLE). The 25(OH)D levels were negatively correlated with the Th17 cell ratio ($r = -0.370$, $p = 0.001$) (Figure 2D).

3.7. Negative correlation between serum 25(OH)D and serum levels of cytokines

The ELISA results showed a significant increase of the levels of IL-23, IL-17, TNF-α, IL-10, and IL-6 in patients with initial-onset childhood SLE (Figures 3A–E, 9.78 ± 4.84 pg/ml vs. 27.53 ± 14.55 pg/ml; 7.80 ± 4.59 pg/ml vs. 12.77 ± 11.00 pg/ml; 3.62 ± 1.55 pg/ml vs.10.32 ± 9.57 pg/ml; 3.66 ± 1.73 pg/ml vs.6.99 ± 5.63 pg/ml; 2.72 ± 1.33 pg/ml vs.18.86 ± 15.98 pg/ml). The serum 25(OH)D levels had a negative correlation with IL-23 ($r = -0.589$, $p < 0.001$), IL-17($r = -0.351$, $p = 0.001$), TNF-α ($r = -0.283$, $p = 0.01$), IL-6 ($r = -0.392$, $p < 0.001$), and IL-10 ($r = -0.313$, $p = 0.004$) levels (Figures 3F–J).

4. Discussion

SLE is a chronic autoimmune disease distinguished by auto-antibodies development and persistent inflammation that damages multiple organs. The clinical manifestations and severity of pediatric SLE are not completely the same as those of adult SLE, with childhood SLE having more severe clinical manifestations and being more prone to involving important organs compared with adult SLE. However, research on childhood SLE has not been widely reported (12).

The increase of Th17 and the decrease of Treg subsets were reported to be the main factors related to organ damages and auto-antibodies production in SLE patients (15). The elevation of the proportion of Th17 cells with pro-inflammatory effects was reported to be positively related to the disease activity of SLE (15). Treg cells have immunosuppressive function and can induce and maintain the self-immune tolerance of the body. The

TABLE 2 Correlations of 25(OH)D levels with the clinical and laboratory parameters.

	R (Spearman correlation)	*p*
24-h urinary protein	−0.423	**<0.001**
C3	0.303	**0.006**
C4	0.225	**0.042**
SLEDAI	−0.168	0.131

Statistically significant results are highlighted in bold.

TABLE 3 Correlations of the Treg ratio with the clinical and laboratory parameters.

	R (Spearman correlation)	*p*
24-h urinary protein	−0.261	**0.018**
C3	0.212	0.056
C4	0.281	**0.011**
SLEDAI	−0.268	**0.015**

Statistically significant results are highlighted in bold.

TABLE 4 Correlations of the Th17 cell ratio with the clinical and laboratory parameters.

	R (Spearman correlation)	*p*
24-h urinary protein	0.277	**0.012**
C3	−0.257	**0.02**
C4	−0.155	0.164
SLEDAI	0.287	**0.009**

Statistically significant results are highlighted in bold.

decrease of Treg and its dysfunction play a very important role in the pathogenesis of SLE (1). Injecting Treg into SLE mice could alleviate inflammation and reduce tissue damage (1). Our study found that the ratio of Th17 cells significantly elevated in initial-onset childhood SLE, while the proportion of Treg significantly decreased compared with healthy controls. However, there have been reports of an increase of the percentage of Treg and Th17 cells rather than a decrease of the number of Treg in SLE patients (2). The research results on the ratio of Treg in SLE patients are inconsistent. Therefore, it is currently believed that not only abnormal proportions but, more importantly, abnormal functions of Treg are involved in the pathogenesis of SLE. Reports confirmed that the mTOR signaling pathway regulates the proliferation, differentiation, and functions of Treg cells (16). Specifically, mTORC1 promotes the expansion of pro-inflammatory lymphocyte subsets such as Th17; mTORC2 drives the proliferation of T follicle helper cells, promoting the activation of B cells and generation of auto-antibodies (17). Both mTORC1 and mTORC2 can control the differentiation and maturation of CD4 + CD25 + Foxp3 + Treg cells (17). In SLE patients, the abnormal metabolism of T cells, including high mTOR activation, increased glutaminolysis, active lipid synthesis, and enhanced glycolysis, all contribute to the differentiation and function of Th17. The metabolic disorder of T cell is a potential mechanism for Th17/Treg imbalance in SLE patients (1).

The serum 25(OH)D levels for children with SLE were obviously lower than those for healthy control children. Consistent with our findings, multiple studies worldwide have found lower levels of 25(OH)D in adults with SLE (10). Although insufficiency and/or deficiency of vitamin D have been reported in children with SLE (11, 12), there is relatively few studies on vitamin D levels in pediatric SLE. There are various reasons for the decrease of serum vitamin D levels in patients

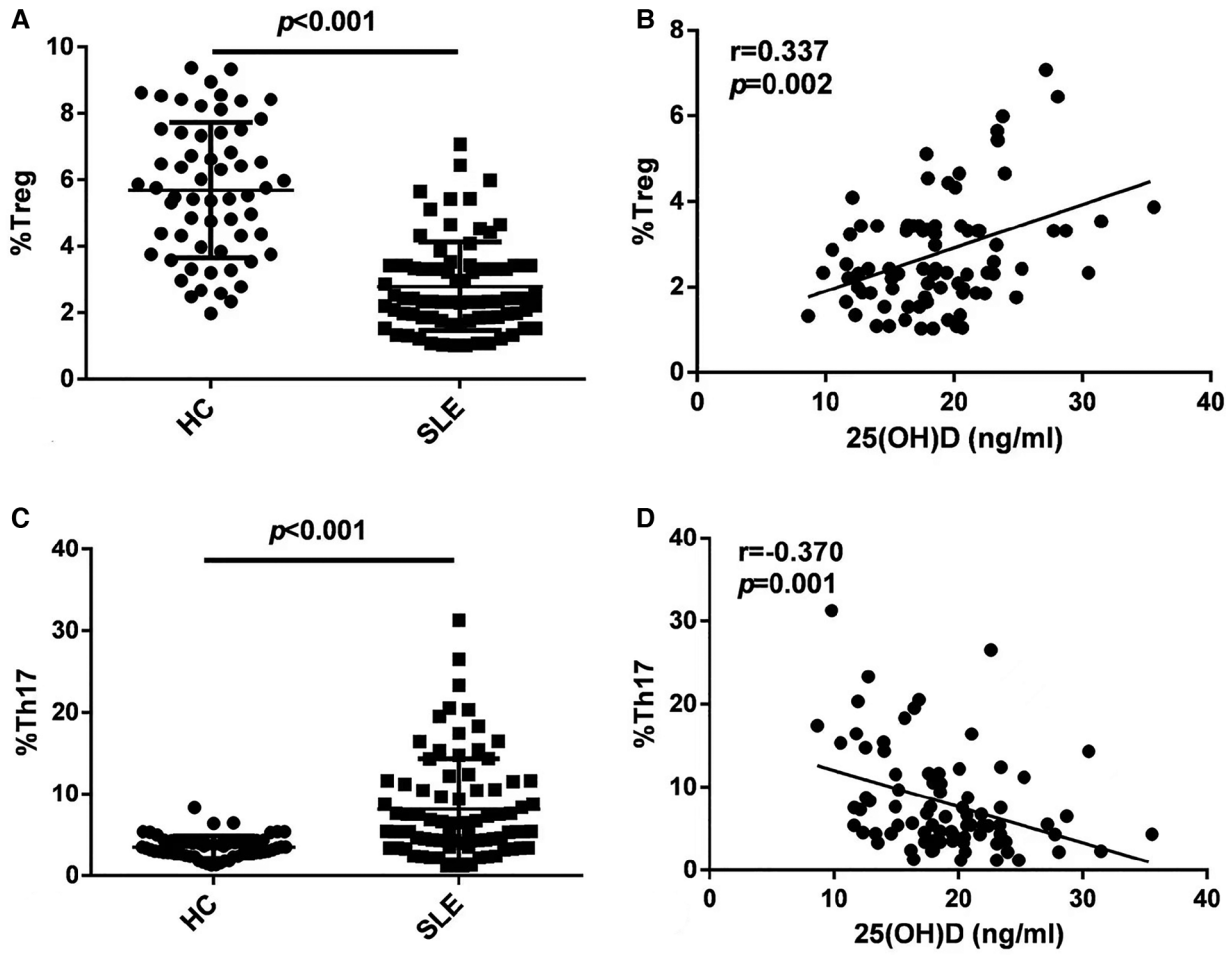

FIGURE 2
Flow cytometry analysis of Treg subset (A) and Th17 cell subset (C) in peripheral blood, *n* = 60 for HC and *n* = 82 for SLE. The correlation analysis of 25 (OH)D levels and Treg subset (B) and Th17 cell subset (D) in peripheral blood in patients with initial-onset childhood SLE (*n* = 82).

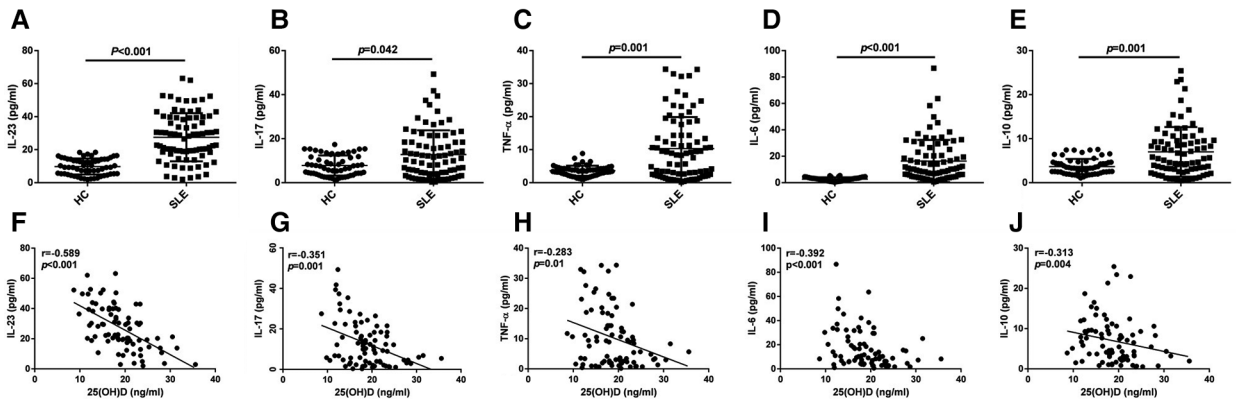

FIGURE 3
ELISA analysis of IL-23, IL-17, TNF-α, IL-6, and IL-10 (**A–E**), *n* = 60 for HC and *n* = 82 for SLE. The correlation analysis of serum 25(OH)D level and IL-23, IL-17, TNF-α, IL-6, and IL-10 in serum from patients with initial-onset childhood SLE (**F–J**) (*n* = 82).

with SLE. Vitamin D is mainly synthesized through the epidermal layer of the skin after ultraviolet exposure (7). The main measures for SLE patients to avoid photosensitivity are sunshade and using sunscreen, but these are also risk factors for vitamin D deficiency (10). Cusack et al. (18) found that using sunscreen had an impact on the levels of 25(OH)D depending on using time. Drugs used to treat SLE may exacerbate vitamin D deficiency, such as glucocorticoids reducing intestinal absorption of vitamin D and accelerating the catabolism of 25(OH)D and l,25$(OH)_2$D by enhancing 24-hydroxylase activity (19, 20). It is reported that proteinuria had a great impact on the concentration of vitamin D, which may be due to the loss of vitamin D-binding protein (DBP) caused by kidney damage in SLE (21). Our study found that the levels of serum 25(OH)D were negatively correlated with the quantification of urinary protein. Young et al. (22) found that vitamin D deficiency was driven by genetic factors, not just due to sun shielding. CYP24A1 rs4809959 modified the association of 25(OH)D and SLE. Vitamin D receptor (VDR) polymorphisms are associated with higher risk of SLE among different races, especially among Asians and Africans (23). Clinical studies found that supplementing vitamin D has an improvement effect on reducing disease activity and alleviating fatigue in patients with SLE (24).

Children with initial-onset SLE had elevated ratios of Thl7 cells and decreased ratios of Treg in their peripheral blood. The levels of 25(OH)D in patients with initial-onset childhood SLE were negatively correlated with the proportion of Th17 cells and positively correlated with the proportion of Treg cells. Th17 cell is a new CD4 + T helper cell subset discovered in recent years. Its proliferation and differentiation are different from Th1 and Th2 cells. Th17 expresses specific nuclear transcription factor rROR γT and can secrete specific cytokines such as IL-17 and IL-22 (25). Vitamin D3 signaling inhibits Th17 cell differentiation. Vitamin D3 acts on Th17 cells, inhibiting the expression of IL-22, IL-17, chemokine receptor CCR6, TNF-α, and IFN-γ, thereby preventing Th17 cells from migrating to inflammatory tissues (26, 27). The 1,25$(OH)_2$D binds the vitamin D receptor to vitamin D response element (VDRE) in the *FoxP3* gene and then directly upregulates the expression of Treg marker *FoxP3* (24). There is evidence to suggest that 1,25$(OH)_2$D can upregulate the expression of *FoxP3* in immature CD4 + T cells and induce differentiation of Treg cells, leading to an increase in the functional expression of regulatory markers such as IL-10 and cytotoxic T lymphocyte-associated protein 4 (CTLA-4) (28). Research studies have also shown that Th2 and Th17 cells are transformed into plastic phenotypes through the action of 1,25 $(OH)_2$D (27). The 1,25$(OH)_2$D induces the phenotype of Treg by upregulating the expression of *FoxP3* and *CLTA4* genes, while downregulating the expression of *IL17A* genes (29).

The levels of cytokines related to Th17 cells, such as IL-6, TNF-α, IL-23, and IL-17, were significantly elevated in patients with initial-onset childhood SLE. The levels of cytokines associated to Treg cells, such as IL-10, were also elevated. The levels of IL-6, TNF-α, IL-23, IL-17, and IL-10 were negatively correlated with the serum 25(OH)D level. Research studies show that after vitamin D treatment, Treg and Th2 cells increased, and Th17 and Th1 cells decreased inconsistently (30, 31). IL-6 inhibits the expression of *Foxp3* during Treg differentiation (32). Relative studies showed that the levels of inflammatory cytokines, such as IL-6, IL-1, IL-18, and TNF-α, were significantly reduced with the vitamin D treatment group in SLE patients. On the other hand, vitamin D treatment upregulated IL-10 expression (33, 34). A study found a positive correlation between elevated serum 25 (OH)D and elevated IL-10. The author showed that after 8 weeks of vitamin D treatment, the levels of IL-10 significantly increased, while there was no significant change in the level of TGF-β1 in multiple sclerosis patients (35). However, our study showed that the levels of IL-10 increased in patients with initial-onset childhood SLE, and a negative correlation can be observed between serum vitamin D levels and blood IL-10 levels, which was inconsistent with other studies.

Vitamin D, as an immune regulatory factor, participates in innate and adaptive immunity (36). The immune regulatory role of vitamin D in autoimmune diseases has always been a focus of research (37). Multiple epidemiological studies worldwide have found vitamin D deficiency or insufficiency in various autoimmune diseases (37).

Vitamin D not only regulates Th17 and Treg cell differentiation, but also acts on other T lymphocyte subsets, B cells, dendritic cells, etc. Both T cells and B cells express VDR, which is an important target for vitamin D to exert immune regulation. Vitamin D induces tolerance phenotype by acting on antigen-presenting cell, monocyte, natural killer cell, and dendritic cells, enhance chemotaxis of neutrophil (38).

Osteoporosis can occur in patients with SLE, including juvenile patients, possibly due to chronic inflammation affecting bone metabolism and the use of glucocorticoids and other drugs (39). It is recommended to monitor the calcium and phosphorus metabolism as well as vitamin D levels in pediatric SLE patients.

Our research has limitations. The sample size included in this study is not large enough and cannot be subjected to a stratified analysis. We only studied Treg and Th17 cells and related cytokines, but did not include other lymphocyte subpopulations. We studied the relation between the serum 25 (OH)D levels and the ratio of Treg and Th17 cells in peripheral blood, but did not conduct a double-blind controlled randomized study to observe the changes in Treg and Th17 cells after vitamin D treatment. The molecular mechanism by which vitamin D acts on Treg and Th17 cells in SLE patients is not yet well understood. These will be explored in our future research.

5. Conclusion

The imbalance of Treg and Th17 cell differentiation leads to the suppression of immune function and promotes the development of SLE. In patients with initial-onset childhood SLE, the changes of serum vitamin D levels can affect the proportion of Treg cell subset and TH17 cell subset and can also affect the levels of cytokines related to these T cell subpopulations. The molecular mechanism of action of vitamin D and lymphocyte subpopulations in SLE is complex. Further exploration should be conducted on the role and mechanism of vitamin D in regulating Th17 and Treg subsets, providing a basis for immunotherapy in pediatric SLE.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving humans were approved by the Ethics Committee of the Second Hospital of Hebei Medical University (protocol number 2021-R307). The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin.

Author contributions

LJ performed the data analyses and wrote the manuscript. ZR contributed significantly to analysis and manuscript preparation. HZ contributed to the conception of the study. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Crescentic glomerulonephritis in children: short-term follow-up predicts long-term outcome

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Background: Crescentic glomerulonephritis (CrGN) is a relatively rare but severe condition in childhood with the clinical feature of rapidly progressive glomerulonephritis (RPGN). The aim of this study is to investigate the clinicopathological features and prognosis of CrGN in children.
Methods: We retrospectively analyzed the clinical and laboratory data, renal pathological results, treatment, and outcome of 147 CrGN in two Chinese pediatric nephrology centers.
Results: Among the 147 children, there were 22 cases of type I (15.0%), 69 cases of type II (46.9%), and 56 cases of type III (38.1%). The mean percentages of crescents in CrGN I, II, and III were 85.3%, 68.7%, and 73.6%, respectively. The children with type I CrGN presented with more severe clinical manifestations and pathological lesions. The 3-month cumulative renal survival rates of types I, II, and III CrGN were 66.3%, 93.6%, and 75.6%, respectively. The 1-year cumulative renal survival rates of types I, II, and III CrGN were 56.9%, 85.3%, and 73.1%, respectively, and the 5-year cumulative renal survival rates of types I, II, and III CrGN were 33.8%, 73.5%, and 47.1%, respectively. The Kappa Consistency Test between the 3-month and 1-year total renal survival (82.1% vs. 74.7%) of the children was 0.683 ($P < 0.001$), and between the 1-year and 5-year total renal-free survival (78.3% vs. 69.1%) of the children was 0.476 ($P < 0.001$). The Bowman's Capsule Rupture (BCR), crescent, interstitial inflammation, and interstitial fibrosis/tubular atrophy (IF/TA) score were predictors of end-stage kidney disease (ESKD) risk but BCR showed better predictive value for ESKD than interstitial inflammation score ($P = 0.027$) and IF/TA score ($P = 0.047$).
Conclusion: Patients with type I tended to have the worst renal survival rates. The three-month renal prognosis could partially reflect the 1-year renal prognosis, and the 1-year mortality rate could partially reflect the 5-year mortality rate of children with CrGN.

KEYWORDS

crescentic glomerulonephritis, pathological lesions, prognosis, end-stage kidney disease, children

Introduction

Crescentic glomerulonephritis (CrGN) is a type of glomerulonephritis that is induced by a variety of causes and result in a sudden and progressive decline in renal function, which is referred to as rapid progressive glomerulonephritis (RPGN). CrGN is defined histopathologically by crescents in 50% or more of glomeruli. CrGN is classified into different subtypes: Type I: anti-glomerular basement-membrane (anti-GBM) disease, which is mediated by anti-glomerular basement membrane (GBM) antibodies, and the

immune complex deposits linearly along the GBM; Type II: CrGN caused by deposition of the immune complex, such as immunoglobulin A nephropathy (IgAN), postinfectious glomerulonephritis (PIGN), lupus nephritis (LN), Henoch-Schonlein purpura nephritis (HSPN), membranoproliferative glomerulonephritis (MPGN); and Type III: pauci-immune glomerulonephritis (little or no staining for immunoglobulins or complement), which is mediated by anti-neutrophil cytoplasmic antibody (ANCA). A few patients show ANCA and anti-GBM antibody double-positive, and clinicians have defined them as type IV. Type V is considered ANCA-negative, pauci-immune glomerulonephritis (5% to 10% of cases) (1). However, the two types in clinic had not been widely accepted.

CrGN is a relatively rare disease; it accounts for 1.6% to 10% of glomerulopathy confirmed by renal biopsy (2). The primary signs of CrGN are hematuria, albuminuria, nephritic sediment, decreased estimated glomerular filtration rate (eGFR), and oliguria. The severity of the disease is related to the percentage of crescent formation. Patients with crescents in more than 80% of glomeruli may not respond well to therapy and tend to present with advanced renal failure. CrGN with a focal lesion with more than 50% normal glomeruli has a more favorable prognosis with almost 90% renal survival after 5 years of follow-up, whereas more than 50% of glomeruli with cellular crescent has a less favorable prognosis (1). However, reports on the prognosis for children with CrGN are scarce. Therefore, we retrospectively analyzed the clinical and pathological features and outcomes of children with CrGN diagnosed by renal biopsy from Jan 2008 to Jan 2018 at two large Chinese diagnostic and treatment centers for children's kidney disease.

Materials and methods

Study design and setting

Children with newly diagnosed CrGN in the Department of Pediatric, Jinling Hospital, Affiliated Hospital of Medical School, Nanjing University and Longgang District Center Hospital of Shenzhen, from Jan 2008 to Jan 2018 were recruited consecutively into the retrospective study. All the patients were <18 years old. The diagnosis was based on an examination of renal biopsy tissue containing at least 10 glomeruli. The ethics committees of Jinling Hospital and Longgang District Center Hospital of Shenzhen approved the study and informed parental consent was obtained. The study conformed to the principles outlined in the Declaration of Helsinki and was approved by the Ethical Committee of Jinling Hospital (number: 2018JLHGKJDWLS-184) and Longgang District Center Hospital of Shenzhen (number: ZSSOM 2018-0177).

Clinical and biochemical measurements

At the time of renal biopsy, baseline data were collected from the hospital's electronic medical record (EMR) system, including age, sex, disease duration, clinicopathological, hematology, and urine examination. The treatment date and pathological features were also obtained from the EMR system. The eGFR was calculated using the modified Schwartz formula (3). Acute kidney injury (AKI), acute kidney disease (AKD), and chronic kidney disease (CKD) definitions were based on the Kidney Disease: Improving Global Outcomes (KDIGO) (4).

Definition

According to the extent of interstitial inflammation, i.e., <1%, 1%–25%, 25%–50%, and >50%, the scores were semi-quantitatively graded as 0, 1, 2, and 3, respectively. For tubulitis, score 1: 0–9 inflammatory cells/tubules, score 2: 10–14 cells/tubules, and score 3: 15-or more cells/tubules in the most affected region (5). The extent of interstitial fibrosis/tubular atrophy (IF/TA) was graded into four categories: score 0: lesion < 5%; score 1: 5% ≤ lesion < 20%; score 2: 20% ≤ lesion < 50%; and score 3: lesion ≥ 50% (6). The extent of sclerosis was evaluated and scored as follows: score 0: <10%, 1: 10–25%, 2: 26–50%, 3: >50%, and the arteriosclerosis lesions were scored as follows: score 0: intimal thickening < thickness of media, score 1: intimal thickening thickness of media) (7).

The segmental crescent volume was less than 50% of the renal capsule, and the spherical crescent volume accounts for more than 50% of the renal capsule. Cellular crescents were defined as cells occupying 50% of the extracapillary lesion, and fibrocellular crescents were defined as extracapillary lesions consisting of cells and extracellular matrix, with cells < 50% and matrix < 90%. A fibrous crescent was defined as > 10% of the capsular perimeter being covered by a lesion composed of ≥ 90% of the matrix. Cellular/fibrocellular/fibrous crescents were calculated according to the relative ratio. Crescent scores = (percent of circumferential cellular/mixed/fibrous crescents) + 1/2(percent of segmental cellular/mixed/fibrous crescents) (8).

Outcome measures

The renal endpoint was end-stage kidney disease (ESKD), and the ESKD-free survival endpoint was defined as death from any cause. ESKD was defined as kidney failure that reaches an eGFR ≤15 ml/min/1.72 m^2 or needs maintaining renal replacement therapy (RRT) for more than 3 months.

Statistical analysis

All analyses were performed using SPSS (version 24.0, SPSS Inc, Chicago, IL, USA). Continuous variables were described as mean and standard deviation or median [interquartile range (IQR)], and differences between groups were analyzed using a two-factor analysis of variance or a non-parametric test. Categorized variables were described as percentages and were analyzed using the χ^2 test. The prognosis was evaluated using Kaplan-Meier curves, and the log-rank test was used to test the two curves' differences. The association of variables with ESKD was assessed with univariate and multivariate Cox proportional hazard regression models. The Kappa

Consistency Test was used to describe the association between the 3-month, 1-year and 5-year outcomes. $P < 0.05$ was considered statistically significant.

Results

Baseline characteristics of children with CrGN

In the present retrospective study, CrGN accounted for 1.1% (147/1, 3,125) of the total number of nontransplant renal biopsies during the study period. Among the 147 patients, 22 (15.0%), 69 (46.9%), and 56 (38.1%) children were classified as type I, type II, and type III, respectively. The patients had a variety of underlying conditions or diseases. The etiology was immune complex in 19 cases, anti-GBM antibody disease in 22 (15.0%) cases, IgAN in 23 (15.7%) cases, LN in 19 (12.9%) cases, HSPN in 14 (9.5%) cases, PIGN in 8 (5.4%) cases, MPGN in 5 (3.4%) cases, ANCA-associated glomerulonephritis (AAGN) in 54 (36.7%) cases, and ANCA-negative pauci-immune CrGN in 2 (1.4%) cases.

The baseline characteristics of the children were presented in Table 1. The mean age was 13.45 ± 3.75 years, and 65 cases (44.2%) were male. The percentage of oliguria or anuria in type II was lower than in type I and type III, respectively ($P < 0.05$, $P < 0.01$). Among the total patients, 11.5% of patients could be classified as stage 1 AKI, 23.7% as stage 2 AKI, and 64.9% as stage 3 AKI. The percentage of stage 3 AKI in type I was higher than in type II and type III, respectively ($P < 0.01$). The levels of procalcitonin (PCT), interleukin-6 (IL-6), and proteinuria in type I were lower than in type II and type III, respectively ($P < 0.05$, $P < 0.01$). The level of serum creatinine (Scr) in type I was higher than in type II and type III, respectively ($P < 0.05$, $P < 0.01$), and eGFR was lower than in type II ($P < 0.01$). The level of urine red blood cell count (RBC) in type III was lower than in type I and type II, respectively ($P < 0.01$). The levels of urine N-acetyl-β-D-glucosidase (NAG) and retinol-binding protein (RBP) in type I were higher than in type II and type III, respectively ($P < 0.01$). Of the patients with type III CrGN, 96.4% were serum ANCA positive. Circulating anti-GBM antibodies were detected in all patients with type I CrGN, among whom 1.4% were positive for both ANCA and anti-GBM. There were 131 (89.1%) patients treated with immunosuppressive therapy, 91 children (61.9%)

TABLE 1 Baseline, demographic and manifestations of children with crescentic glomerulonephritis.

Variables	Total ($n = 147$)	Type I ($n = 22$)	Type II ($n = 69$)	Type III ($n = 56$)	*P* value
Age (years)	13.45 ± 3.75	13.21 ± 4.03	12.76 ± 3.41	14.19 ± 3.87	>0.05
Gender [males, *n* (%)]	65 (44.2)	13 (59.1)	36 (52.2)	16 (28.6)	>0.05
Hypertension	22 (15.0)	2 (9.1)	16 (23.2)	4 (7.1)	0.015[c]
Oliguria or anuria	120 (81.6)	20 (90.9)	48 (69.6)	52 (92.9)	0.045[a], 0.001[c]
AKI, *n* (%)	131 (89.1)	22 (100.0)	60 (87.0)	49 (87.5)	>0.05
AKI 1 stage, *n* (%)	15 (11.5)	0 (0.0)	8 (11.6)	7 (12.5)	>0.05
AKI 2 stage, *n* (%)	31 (23.7)	2 (9.1)	19 (27.5)	10 (17.9)	>0.05
AKI 3 stage, *n* (%)	85 (64.9)	20 (90.9)	33 (47.8)	32 (57.1)	<0.001[a], 0.004[b]
PCT, μg/L	0.68 (0.44, 0.84)	0.45 (0.18, 0.67)	0.70 (0.55, 0.81)	0.88 (0.54, 1.02)	0.043[a], 0.008[b]
IL-6, ng/L	29.72 ± 6.24	22.07 ± 5.82	32.07 ± 6.24	41.44 ± 5.92	0.037[a], 0.041[b]
Scr (mmol/L)	354.37 (78.52, 1,033.57)	536.44 (176.38, 1,033.57)	223.06 (76.19, 347.21)	338.56 (127.32, 426.06)	0.013[a], 0.041[b]
eGFR, mL/min/1.73 m^2	53.45 (16.00, 78.02)	25.07 (13.00, 48.02)	67.14 (24.43, 80.11)	47.38 (27.48, 67.04)	0.000[a]
Low C3	73 (49.7)	7 (31.8)	36 (52.2)	23 (41.1)	>0.05
Proteinuria (mg/kg•24 h)	36.52 ± 6.53	18.72 ± 4.04	43.36 ± 8.58	29.84 ± 5.54	<0.001[a], 0.003[b]
Urine RBC, ul/ml	778.00 (275.00, 2,100.00)	1,100.00 (775.00, 2,300.00)	850.00 (277.00, 5,487.50)	424.50 (228.75, 1,025.25)	<0.001[b], <0.001[c]
Urine NAG, U/g*cr	48.14 (31.10, 96.78)	86.10 (45.80, 106.93)	46.68 (36.24, 55.50)	38.60 (24.60, 72.10)	<0.001[a], <0.001[b]
Urine RBP, mg/L	11.44 (3.00, 28.55)	29.00 (27.53, 31.32)	11.44 (3.26, 26.64)	7.80 (12.35, 26.32)	<0.001[a], <0.001[b]
Anti-GBM positive, *n* (%)	24 (14.9)	22 (100.0)	0 (0.0)	2 (3.6)	–
ANCA positive, *n* (%)	61 (41.5)	2 (9.1)	5 (7.3)	54 (96.4)	<0.001[b], <0.001[c]
MPO-ANCA positive, *n* (%)	58 (39.5)	2 (9.1)	5 (7.3)	51 (91.1)	<0.001[b], <0.001[c]
PR3-ANCA positive, *n* (%)	5 (3.4)	0 (0.0)	0 (0.0)	5 (8.9)	–
Treatment					
Immunosuppressive therapy, *n* (%)	131 (89.1)	22 (100.0)	58 (84.1)	51 (91.1)	>0.05
CRRT, *n* (%)	91 (61.9)	19 (86.4)	27 (39.1)	45 (80.4)	<0.001[a], <0.001[c]
ESKD three-month	13 (8.8)	5 (22.7)	3 (4.4)	4 (7.1)	0.008[a]
ESKD one-year	18 (12.2)	7 (31.8)	4 (5.8)	7 (12.5)	0.001[a], 0.045[b]
ESKD five-year	53 (36.7)	11 (50.0)	17 (24.6)	26 (44.6)	0.025[a], 0.011[c]
Dead five-year	7 (4.8)	3 (13.6)	2 (2.9)	2 (3.6)	>0.05

AKI, acute kidney injury; PCT, procalcitonin; IL-6, interleukin-6; Scr, serum creatinine; eGFR, estimate glomerular filtration rate; RBC, red blood cell count; NAG, N-acetyl-β-D-glucosidase; RBP, retinol-binding protein; Anti-GBM, anti-glomerular basement membrane; ANCA, antineutrophil cytoplasmicantibody-associated glomerulonephritis; CRRT, continuous renal replacement therapy; ESKD, end stage kidney disease.
Scr, AKI acute kidney injury.
[a]$P < 0.05$ between types I and II.
[b]$P < 0.05$ between types I and III.
[c]$P < 0.05$ between types II and III.

received continuous renal replacement therapy (CRRT) treatment, and the percentage of CRRT treatment in type II was lower than in type I and type III, respectively ($P < 0.01$) (Table 1).

Pathological characteristics

Among the subtypes of CrGN, the scores for crescents, sclerosis, interstitial inflammation, and IF/TA in type II were lower than in type I and type III, respectively ($P < 0.05$, $P < 0.01$). The percentage of cellular crescentic in type I was higher than in type II and type III, respectively ($P < 0.05$, $P < 0.01$), and the fibrotic crescentic in type II was higher than in type I and type III, respectively ($P < 0.05$, $P < 0.01$). The tubulitis and arteriosclerosis scores in type III were higher than in type I and type II, respectively ($P < 0.05$, $P < 0.01$). Importantly, Bowman's Capsule Rupture (BCR) presented in 62 children (42.2%) with CrGN, and BCR in type I was higher than in type II and type III, respectively ($P < 0.05$, $P < 0.01$) (Table 2).

Prognostic factors of renal outcome

We conducted Cox regression analyses to explore the clinical, laboratory, or histologic factors associated with ESKD. As shown in Table 3, multivariate analysis demonstrated that crescent score (HR = 2.185, 95% CI: 0.745–3.927, $P = 0.033$), interstitial inflammation score (HR = 5.164, 95% CI: 2.745–16.827, $P = 0.037$), IF/TA score (HR = 1.750, 95% CI: 1.018–3.009, $P = 0.029$), and BCR (HR = 8.874, 95% CI: 2.116–24.291, $P = 0.012$) were predictors of ESKD risk. The area under the curve (AUC) for ESKD prediction at the end of follow-up for the crescent score, interstitial inflammation score, IF/TA score, and BCR was 0.709 ($P = 0.008$), 0.657 ($P = 0.031$), 0.556 ($P = 0.045$), and 0.812 ($P < 0.001$), respectively. The best cutoff value for crescent score, interstitial inflammation score and IF/TA score were 2.45, 2.50 and 1.50, respectively, and the sensitivity and specificity of crescent score, interstitial inflammation score, IF/TA score and BCR were 66.7%, 77.8%, 55.6%, 100.0% and 71.7%, 51.5%, 73.2%, 62.3%, respectively. BCR showed better predictive value for ESKD than the interstitial inflammation score ($P = 0.027$) and IF/TA score ($P = 0.047$). However, there was no significant difference between the other factors ($P > 0.05$) (Figure 1).

Renal survival in short- and long-term

As shown in Figure 2, the 3-month cumulative renal survival rate for types I, II, and III CrGN was 66.3%, 93.6%, and 75.6%, respectively, and the rate in type I was significantly lower than in type II ($P < 0.001$). The 1-year cumulative renal survival rate for types I, II, and III CrGN was 56.9%, 85.3%, and 73.1%, respectively,

TABLE 2 Renal pathology data by type of crescentic glomerulonephritis.

Variables	Total ($n = 147$)	Type I ($n = 22$)	Type II ($n = 69$)	Type III ($n = 56$)	P value
Crescent score	1.81 ± 0.56	2.27 ± 0.49	0.96 ± 0.31	2.04 ± 0.52	0.008[a], 0.012[c]
Cellular crescentic	25.00 (11.33, 49.17)	40.50 (34.58, 47.08)	21.37 (12.50, 56.47)	27.27 (12.78, 43.46)	0.007[a], 0.044[b]
Fibrotic crescents	21.00 (9.85, 31.39)	28.50 (19.43, 43.81)	12.00 (7.00, 21.50)	22.50 (13.50, 33.75)	<0.001[a], 0.022[c]
Sclerosis score	1.34 ± 0.56	1.37 ± 0.38	0.84 ± 0.37	1.54 ± 0.45	<0.001[a], 0.004[c]
Bowman's capsule rupture, n (%)	62 (42.2)	15 (63.6)	23 (47.7)	24 (41.1)	0.004[a], 0.044[b]
Interstitial inflammation score	1.66 ± 0.30	1.57 ± 0.32	0.94 ± 0.22	1.83 ± 0.25	0.036[a], 0.041[c]
Tubulitis score	1.36 ± 0.52	1.22 ± 0.46	0.85 ± 0.32	1.56 ± 0.34	0.005[c]
TA/IF score	1.14 ± 0.22	1.18 ± 0.24	0.98 ± 0.18	1.36 ± 0.31	0.009[a], 0.018[c]
Arteriosclerosis score	1.05 ± 0.36	0.98 ± 0.31	0.86 ± 0.24	1.25 ± 0.44	0.014[c]

IF/TA, intersititial fibrosis/tubular atrophy.
[a]$P < 0.05$ between types I and II.
[b]$P < 0.05$ between types I and III.
[c]$P < 0.05$ between types II and III.

TABLE 3 Risk factors of children progression to ESKD with CrGN.

Variables	Univariate analysis		Multivariate analysis	
	OR (95% CI)	P	OR (95% CI)	P
Scr (≥110 μmol/L)	10.237 (2.281–29.146)	0.048		
eGFR (≤15 ml/min/1.73 m^2)	2.432 (1.326–14.758)	0.076		
CRRT	5.164 (1.033–8.673)	0.058		
PE	8.114 (0.152–17.146)	0.024		
Crescent score (per 0.01 increased)	3.225 (0.894–4.259)	0.011	2.185 (0.745–3.927)	0.033
Interstitial inflammation score (per 0.01 increased)	6.395 (3.064–18.191)	0.018	5.164 (2.745–16.827)	0.037
IF/TA score (per 0.01 increased)	2.386 (1.328–5.198)	0.007	1.750 (1.018–3.009)	0.029
Bowman's capsule rupture	12.352 (3.768–32.719)	0.002	8.874 (2.116–24.291)	0.012

Scr, serum creatinine; eGFR, estimate glomerular filtration rate; CRRT, continuous renal replacement therapy; PE, plasma exchange; IF/TA, intersititial fibrosis/tubular atrophy; BCR, Bowman's capsule rupture.

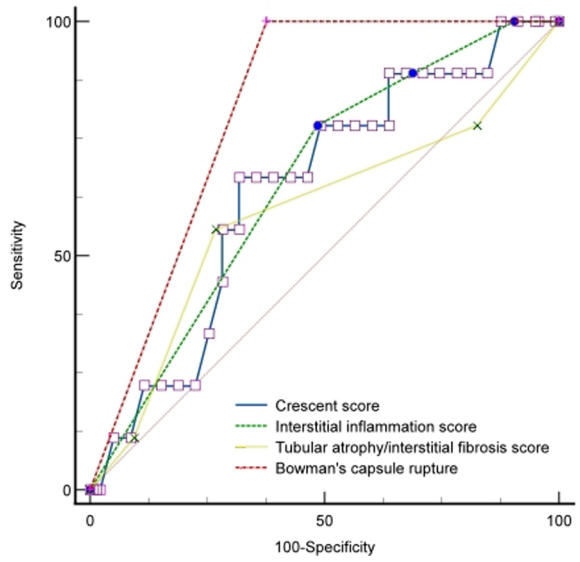

FIGURE 1
ROC curve of renal outcomes with the BCR, crescent, interstitial inflammation, and IF/TA scores.

and the rate in type I was significantly lower than in types II and II ($P < 0.005$, $P < 0.001$). The 5-year cumulative renal survival rate for types I, II, and III CrGN was 33.8%, 73.5%, and 47.1%, respectively, and compared with types I and III, type II of CrGN had a better renal recovery ($P < 0.001$) (Figure 3). The Kappa Consistency Test between the 3-month and 1-year total renal survival (82.1% vs. 74.7%) of the children was 0.683 ($P < 0.001$). After the 5-year follow-up, 7 children were dead, 3 children in types I and 3 children each in types II and III. The 5-year cumulative renal-free survival rate for types I, II, and III CrGN was 63.6%, 70.0%, and 69.6%, respectively, and the rate in type I was significantly lower than in type II ($P < 0.005$) (Figure 1). Within the 3-month follow-up, there were no deaths in the three types, and the Kappa Consistency Test between 1-year and 5-year total renal-free survival (78.3% vs. 69.1%) of the children was 0.476 ($P < 0.001$).

Discussion

Validation studies of epidemiological data on CrGN had been performed in many countries, including Germany, Italy, Sweden,

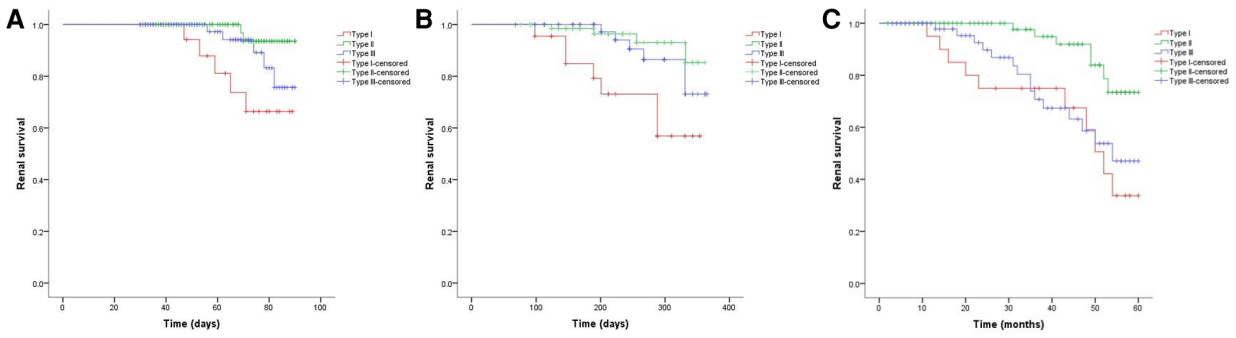

FIGURE 2
Cumulative renal survival of patients with different types of crescentic glomerulonephritis at 3-months (A), 1-year (B), and 5-year (C) follow-ups.

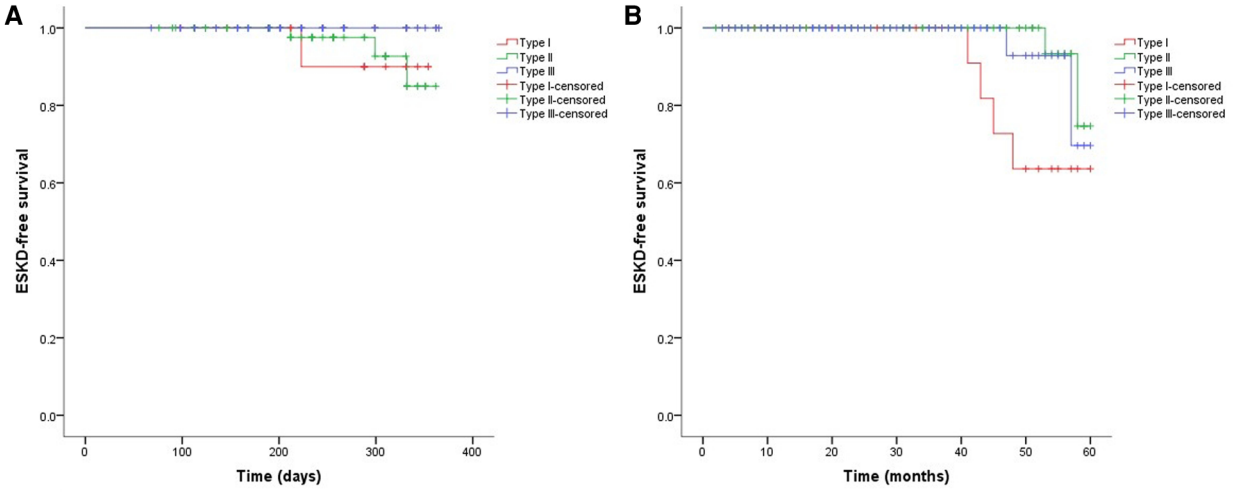

FIGURE 3
Cumulative ESKD-free survival in different types of crescentic glomerulonephritis at 1-year (A) and 5-year (B) follow-ups.

Turkey, Japan, and India, showing that CrGN accounted for 2.65% to 13% of total renal biopsies (9–13), and the date was 1.6%–3.7% in China (2, 14–16). There have been few reports of CrGN in children with large cohorts in the last 10 years (17–21). The 147 children with CrGN in our study accounted for 1.1% (147/1, 3,125, 1.1%) of renal biopsies in children during the same period, which was lower than in previous reports. Unlike adult studies in which type III CrGN was the most common histopathological form, immune complex glomerulonephritis was the most common cause of CrGN in children, commonly secondary to PIGN, HSPN, and IgAN. IgAN (15.6%) was the most common cause of type II CrGN, followed by LN (12.9%) and HSPN (9.5%) in our cohort. This small difference in epidemiological and etiology data may be related to the fact that our two institutes were tertiary care hospitals, and genetic and local factors may also affect the prevalence of the disease.

Of all the patients, type I CrGN showed more severe clinical manifestations than the other types, including AKI, PCT, IL-6, Scr, and eGFR. We also found different renal pathologic characteristics among the three subtypes. The scores for crescents, interstitial inflammation, and IF/TA in type II were lower than in type I and type III, respectively. Importantly, the percentage of BCR in type I was higher than in type II and type III. Multivariate analysis demonstrated that the crescent score, interstitial inflammation score, IF/TA score, and BCR were predictors of ESKD risk. A strong predictor of the outcome for all types of CrGN was the severity of kidney pathological lesions. Chen. et al. reported that pathological severity, represented by a higher crescent score, was associated with long-term outcomes (6). Zhao et al. found that acute tubular necrosis and Bowman's Capsule (BC) membrane thickening at presentation were independent risk factors for ESKD (22). In a South Asia survey, IF/TA and percentage of crescents were significant negative prognostic factors for patients with CrGN survival in the long term (23). Özlü. et al. demonstrated that the ratio of fibrous and/or fibrocellular crescents was inversely correlated with the response to treatment and development of ESKD (18), and fibrinoid necrosis and IF/TA were reported to be important risk factors for renal prognosis (24). Recently, histopathologic classification, chronic inflammation, and histopathologic vascular changes have been identified as a predictor for renal outcomes among CrGN patients (25, 26). Importantly, BCR showed better predictive value for ESKD than interstitial inflammation and the IF/TA score, and BCR was observed in 42.2% of children with CrGN, which demonstrates the importance of BCR in the pathological diagnosis and prognosis evaluation in CrGN and might be ignored in clinicopathology.

In the case of systemic and kidney-restricted diseases, the glomerular capillaries could develop lesions and necrosis, resulting in ruptures of the GBM and fibrin exudation. This process also triggers the activation of cellular and humoral components of inflammation in the BC, leading to the proliferation of parietal epithelial cells. The proliferation narrows the remaining space in the BC and presents as crescents on renal biopsy. BCR is the final step in crescent formation, and the proliferation of parietal epithelial cells during crescent formation leads to BCR (27). An intact BC prevents inflammatory cells from gaining access to the glomerular space. However, once the BC is breached, inflammatory cells could access the glomerular space in CrGN with BCR enabling direct pathological interaction between both compartments. When renal inflammation leads to the destruction of glomerular capillaries and the release of pro-inflammatory cytokines and chemokines into the BC, parietal epithelial cells proliferate and build new crescents (28). As the characteristic performance of CrGN, crescent formation has long been recognized as an indicator of the severity of the inflammatory process affecting the glomerulus. Our study also suggested that interstitial inflammation was a predictor of ESKD risk, which, accompanied by BCR, could be potential therapeutic targets.

In our cohort study, type I CrGN received more CRRT than type II and III, but the prognosis was not improved. Although prompt and intensive treatment measures were taken, including immunosuppressive therapy, CRRT, and plasma exchange (PE), 36.7% of the children progressed to ESKD at the 5-year follow-up. As CKD was defined as abnormalities of kidney structure or function, presented for >3 months (4), the endpoint of many AKI studies was 60- or 90-day mortality (29). ESKD at 1 year was a clinically important short-term outcome measure in patients with glomerulonephritis (GN) (30), and more than half of children with GN and crescents who progressed to ESKD did so within the first year after initial biopsy (17, 21). Therefore, we observed the short- and long-term renal and renal-free survival at 3-month, 1-year, and 5-year follow-ups, and the results showed that the Kappa Consistency Test between the 3-month and 1-year renal prognosis was 0.683 ($P < 0.001$), which indicated that the 3-month renal prognosis could partially reflect the 1-year renal prognosis. The result of the Kappa Consistency Test between 1-year and 5-year renal-free survival was 0.476 ($P < 0.001$), which indicated that the 1-year mortality rate could partially reflect the 5-year mortality rate of children with CrGN. From the association between 3-month and 1-year renal prognosis, we can infer that clinicians could predict early renal prognosis in children with CrGN, and aggressive therapy within 3 months was important for the recovery of renal function.

Limitations of this study: first, this study was a retrospective study of cases from two centers, and the trajectories identified in these participants may not be generalizable to other populations. Second, due to the limitations of retrospective studies, the choice of treatment may weaken the rigor of the study.

Conclusions

In conclusion, AAGN, IgAN, and LN were the most common pathological types in children with CrGN in our cohort. The clinical manifestations, pathological change, and prognosis varied among different CrGN types. BCR, crescent, interstitial inflammation, and IF/TA scores were risk factors for the disease progressing to ESKD. The 3-month renal prognosis could

partially reflect the 1-year renal prognosis, and the 1-year mortality rate could partially reflect the 5-year mortality rate of children with CrGN.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding authors.

Ethics statement

The studies involving human participants were reviewed and approved by Ethical Committee of Jinling Hospital and Longgang District Center Hospital of Shenzhen. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

Research idea and study design: PZ; data acquisition and statistical analysis: WW and XY; supervision or mentorship: C-lG and Z-kX. All authors discussed the results and contributed to the final manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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CCL22 and Leptin associated with steroid resistance in childhood idiopathic nephrotic syndrome

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Objective: Previous studies have indicated a decrease in T regulatory cells (Tregs) among patients with steroid-resistant nephrotic syndrome. CCL22 and Leptin influenced the immune function of Tregs through their respective pathways. This study aimed to compare patients with steroid-sensitive nephrotic syndrome (SSNS) and steroid-resistant nephrotic syndrome (SRNS) in terms of CCL22 and Leptin levels.

Methods: This prospective study included 117 children diagnosed with idiopathic nephrotic syndrome (INS). Peripheral blood samples were collected before initiating steroid therapy, and serum levels of CCL22 and Leptin were measured. Patients were categorized into three groups based on their response to steroid treatment. Renal biopsies were recommended for all children diagnosed with INS, with higher acceptance rates in glucocorticoid resistance patients.

Results: Based on the response to steroid treatment, 117 children were divided as groups of SSNS (82 cases), frequent relapse nephrotic syndrome (FRNS) (10 cases), and SRNS (25 cases). A total of 41 patients underwent kidney biopsy, 11 cases (13.4%) in SSNS, 7 cases (70.0%) in FRNS and 24 cases (96.0%) in SRNS. 30 cases were minimal change disease (MCD), 9 cases were mesangial proliferative glomerulonephritis (MsPGN) and 3 cases were focal segmental glomerulosclerosis (FSGS). The levels of Leptin were significantly higher in SR patients (1208.1 ± 1044.1 pg/ml) compared to SS patients (515.4 ± 676.9 pg/ml) and controls (507.9 ± 479.8 pg/ml), regardless of the pathological type. CCL22 levels were significantly elevated in SRNS (92.2 ± 157.0 pg/ml), but the difference seemed to be attributed to the specific type of pathology, such as Minimal change disease (MCD) (127.4 ± 206.7 pg/ml) and focal segmental glomerulosclerosis (FSGS) (114.8 ± 22.0 pg/ml). For SRNS prediction, the AUC of Leptin, CCL22, and the joint prediction index were 0.764, 0.640, and 0.806, respectively.

Conclusion: Serum levels of CCL22 and Leptin, detected prior to steroid therapy, were associated with steroid resistance in childhood INS.

KEYWORDS

CCL22, leptin, steroid-resistant nephrotic syndrome, T regulatory cells, idiopathic nephrotic syndrome

Introduction

Steroid-resistant nephrotic syndrome (SRNS) is characterized by the lack of response to steroid therapy, resulting in persistent proteinuria after four weeks of standard treatment (1). Among children diagnosed with nephrotic syndrome, approximately 10%–20% are clinically identified as having SRNS. Within five years of diagnosis, nearly 50% of children with SRNS

progress to end-stage renal disease (2). The clinical manifestations of SRNS in children exhibit high heterogeneity, and its progression is primarily assessed through invasive pathological examination of kidney biopsies. The management of SRNS remains a significant challenge for pediatric nephrologists, and currently, there is no widely accepted tool for early prediction of steroid therapy resistance.

Studies have demonstrated an association between low steroid responsiveness and the regulation of T lymphocyte function. Patients with SRNS exhibit a decrease in T regulatory cells (Tregs), whereas an increase in Tregs has been observed in response to effective immunosuppressive or monoclonal antibody therapies (3). Tregs play a critical role in immunoregulation and exert immunosuppressive effects through various cellular and molecular mechanisms. They suppress CD4+ and CD8+ T cells, dendritic cells (DCs), B cells, natural killer (NK) cells, and macrophages (4).

Leptin and CCL22 have been identified as potential immunomodulators due to their interactions with Tregs. Leptin has been shown to sustain the activity of pro-inflammatory cytokines and immune cells, while also enhancing the immune response by stimulating M2 macrophages, promoting Th1 and Th17 cells, and inhibiting Tregs (5, 6). Inhibiting the Leptin pathway has been found to preserve Tregs proliferation and alleviate symptoms in certain autoimmune diseases (7). CCL22, a macrophage-derived immunosuppressive chemokine, acts through the CCL22-CCR4 axis to recruit Tregs. This occurs mainly in the secondary lymphoid organs and is eminently important for the control of adaptive immunity. Therefore, CCL22 most likely represents a central immune checkpoint that controls T-cell immunity (8).

In order to examine the involvement of Leptin and CCL22 in the development of SRNS, this study prospectively obtained serum samples from children with nephropathy prior to commencing steroid therapy. The patients were classified based on their response to steroid treatment, and the relationship between serum Leptin and CCL22 levels and steroid sensitivity in children with nephrotic syndrome was analyzed.

Materials and methods

Population

This study was conducted from January 2019 to September 2021 and involved children with idiopathic nephrotic syndrome who had not received prior steroid therapy. The study protocol involving human participants was reviewed and approved by the Ethics Committee.

The diagnosis of idiopathic nephrotic syndrome (INS) was based on the presence of edema, 24-h urinary protein excretion of ≥50 mg/kg, morning urinary protein/creatinine of >2 mg, hypoalbuminemia of <25 g/L and the disease of unknown causing. All children with INS received the standard steroid therapy and were classified into three categories, steroid-sensitive nephrotic syndrome (SSNS), frequent relapse nephrotic syndrome (FRNS) and steroid-resistant nephrotic syndrome (SRNS), on the basis of their clinical responses toward steroids. The SSNS group included patients with negative urinary protein for ≤4 weeks in those treated with sufficient prednisone [2 mg/(kg·d) or 60 mg/(m·d)]. The frequent relapse group included patients in whom INS recurred two times or more within half a year, or four times or more within 1 year in the course of the disease. The SRNS group included patients who failed to achieve remission after 4 weeks of daily sufficient prednisone. FRNS and SRNS were collectively referred to as refractory nephrotic syndrome (RNS). Further, the relapse group included patients in whom the quantity of urinary protein was ≥50 mg/kg, or the urinary protein/creatinine (mg/mg) of morning urine was ≥2.0, or the morning urinary protein changed from negative to positive for three consecutive days. The non-relapse group included patients in whom INS no recurred within 1 year after the first complete remission. The infrequent relapse group included patients in whom INS recurred once within 6 months or one to three times within 1 year after the first complete remission. Patients who were not finished four weeks of glucocorticoid therapy, or received other immunosuppressants, monoclonal antibodies, or cytotoxic drugs within four weeks, would be excluded from the study.

The clinical features of enrolled patients were recorded from the medical record, such as age, genders, creatinine, estimated glomerular filtration rate (eGFR), 24-h urine protein (Upro), occurred of complications and hypertension. eGFR was calculated according to Schwarz-formula (9).

Sample collection

The blood sample were collected into vacuum sampling vessel containing coagulant (Improve Medical, China) before steroid therapy from the enrolled patients. Serum were separated within 2 h after blood collection and then divided and stored in the refrigerator at 2–8°C immediately. All the serum samples were transferred to an ultra-low temperature refrigerator (−80°C) no more than 8 h. The samples were guaranteed not to thaw until the ELISA test.

Evaluation of renal biopsies

Renal biopsies were recommended in patients with INS diagnosis. Pathology test should obtain the written informed consent of the guardian first. Renal biopsies were executed by skilled nephrologists, and all biopsies were assessed by pathologists through light microscopy and immunofluorescence. Each kidney sample was observed for the total number of glomeruli, glomerular sclerosis, mesangial proliferation, basement membrane thickening, tubular degeneration and atrophy, interstitial fibrosis, interstitial inflammation, etc.

ELISA tests

Human Leptin ELISA KIT (4A biotech, China, Assay range: 31.25–2000 pg/ml), Human CCL22 ELISA KIT (4A biotech,

China, Assay range: 15.625–1000 pg/ml) were used for patient serum test. Briefly, to assay each protein, serum samples at an optimal dilution were added to a microplate precoated with capture antibody, incubated, washed and followed by addition of capture antibody, horseradish peroxidase and substrate. The absolute levels of serum protein biomarkers were determined using standard curves run on each ELISA plate, and normalized by urine creatinine concentration.

Statistical analysis

The quantitative data with normal distribution was expressed as (Average ± SD), and the classified data indicated with the quantity of each component separately. Statistical analysis was performed using SPSS 22.0. The chi-square test was used to analyze gender differences among the groups, while the *t*-test was used to assess age differences. Outlier detected by histogram, and replaced with the average in statistical process. Pearson bivariate correlation analysis was used for correlation statistics. A *P*-value greater than 0.05 indicated no significant difference. After quantifying the ELISA results based on the standard curve, the *t*-test or Wilcoxon rank sum test was used for between-group comparisons, and analysis of variance or Kruskal-Wallis test was used for comparisons involving more than two groups. Statistical significance was set at $P < 0.05$. Receiver operating characteristic (ROC) curves were utilized to determine the effective area, sensitivity, and specificity of candidate indexes.

Results

Clinical features

A total of 117 cases (86 males, 31 females) with childhood INS were included in the study, comprising the subgroups of SSNS (82 cases), FRNS (10 cases), and SRNS (25 cases). Additionally, 40 cases (28 males, 12 females) undergoing health check-ups and confirmed to be free of obvious diseases were selected as the healthy control group. Serum samples were collected before steroid therapy and stored according to the aforementioned protocol. The clinical features of children enrolled in the study were listed in Table 1. There were no significant differences in age, gender ratio, Creatinine, eGFR and Upro between the three groups ($P > 0.05$). There were 148 complications could be recorded in 117 INS patients, with 12 types. The first three kinds were inflammation (48 cases), hydrops (30 cases) and liver injury (23 cases). There was no difference in the incidence of complications among three subgroups ($P > 0.05$). The incidence of hypertension was different ($P < 0.05$), with the highest in SRNS.

A total of 41 (35.0%) patients underwent kidney biopsy, 11 cases (13.4%) in SSNS, 7 cases (70.0%) in FRNS and 24 cases in SRNS with an acceptance rate up to 96.0%. Minimal change disease (MCD) was the most common pathological type with 30 cases, mesangial proliferative glomerulonephritis (MsPGN) with 9 cases and focal segmental glomerulosclerosis (FSGS) with 3 cases. Pathological types in the three groups with serum concentration of CCL22 and Leptin were shown in Table 2.

Leptin and CCL22 had no significant correlation with clinical features

There was no significant correlation between Leptin and Creatinine ($P = 0.368$), eGFR ($P = 0.329$), Upro ($P = 0.241$). At the same between CCL22 and Creatinine ($P = 0.060$), eGFR ($P = 0.570$), Upro ($P = 0.963$). These indicated that changes of Leptin and CCL22 in INS patients had a different significance compared to clinical features.

Leptin levels were elevated in RNS, especially in cases of SRNS

The concentration of serum Leptin was significantly increased in RNS, with even higher levels observed in the SRNS subgroup. No significant differences were found between the healthy group and SSNS. The results were depicted in Figure 1A. Patients in SSNS were distinguished as no relapse and infrequent relapse with fewer recurrences than FRNS. Leptin was associated with the frequency of relapse, and patients with FRNS had significantly elevated Leptin concentrations (Figure 1B). Regarding the

TABLE 1 The clinical features of children enrolled in the study.

Clinical features	INS				Healthy	*P*-value
	Total	SSNS	FRNS	SRNS		
Cases, *n*	117	82	10	25	40	/
Age, months	48.5 ± 36.4	46.1 ± 33.3	45.3 ± 23.2	57.4 ± 47.5	66.1 ± 17.8	0.389
Genders, male/female	86/31	63/19	7/3	16/9	28/12	0.607
Creatinine, μmol/L	39.9 ± 13.9	39.4 ± 13.7	39.9 ± 3.99	41.2 ± 16.6	/	0.860
eGFR, ml/min/1.73 m^2	130.0 ± 42.9	132.4 ± 45.6	119.2 ± 20.4	126.5 ± 39.4	/	0.600
24-h urine protein, mg/24 h	2526.7 ± 2803.8	2225.2 ± 2242.8	2219.6 ± 1277.4	3797.9 ± 4282.9	/	0.065
Complications, numbers/per capite	148/1.26	104/1.27	10/1.00	34/1.36	/	0.838
Hypertension, *n* (%)	8 (6.8%)	2 (2.4%)	1 (10.0%)	5 (20.0%)	/	**0.009**

SSNS, steroid-sensitive nephrotic syndrome; FRNS, frequent relapse nephrotic syndrome; SRNS, steroid-resistant nephrotic syndrome, *P*-value: <0.05 means statistically significant difference; eGFR, estimated glomerular filtration rate.
The bold values mean $P < 0.05$ with the significant difference.

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TABLE 2 Pathological types in three groups with serum concentration of CCL22 and Leptin.

Groups	Pathological types	Cases	Leptin (pg/ml)	*P*-value to MsPGN	CCL22 (pg/ml)	*P*-value to MsPGN
SSNS						
	MsPGN	1	377.8	/	24.2	/
	MCD	10	1139.1 ± 1221.1	/	36.6 ± 36.4	/
FRNS						
	MCD	7	1201.7 ± 1247.6	/	17.7 ± 5.4	/
SRNS						
	MsPGN	8	1320.9 ± 1189.8	/	26.6 ± 15.3	/
	MCD	13	1206.5 ± 1067.1	0.822	73.2 ± 70.2	0.189
	FSGS	3	914.6 ± 753.9	0.601	114.8 ± 22.0	**<0.001**
Total		42	1200.2 ± 1089.4	/	67.2 ± 122.7	/
	MsPGN	9	1216.1 ± 1156.5	/	26.3 ± 14.4	/
	MCD	30	1253.0 ± 1204.8	0.984	49.9 ± 55.4	0.086
	FSGS	3	914.6 ± 753.9	0.624	114.8 ± 22.0	**<0.001**

SSNS, steroid-sensitive nephrotic syndrome; FRNS, frequent relapse nephrotic syndrome; SRNS, steroid-resistant nephrotic syndrome; MCD, minimal change disease; MsPGN, mesangial proliferative glomerulonephritis; FSGS, focal segmental glomerular sclerosis; *P*-value: <0.05 means statistically significant difference.
The bold values mean $P < 0.05$ with the significant difference.

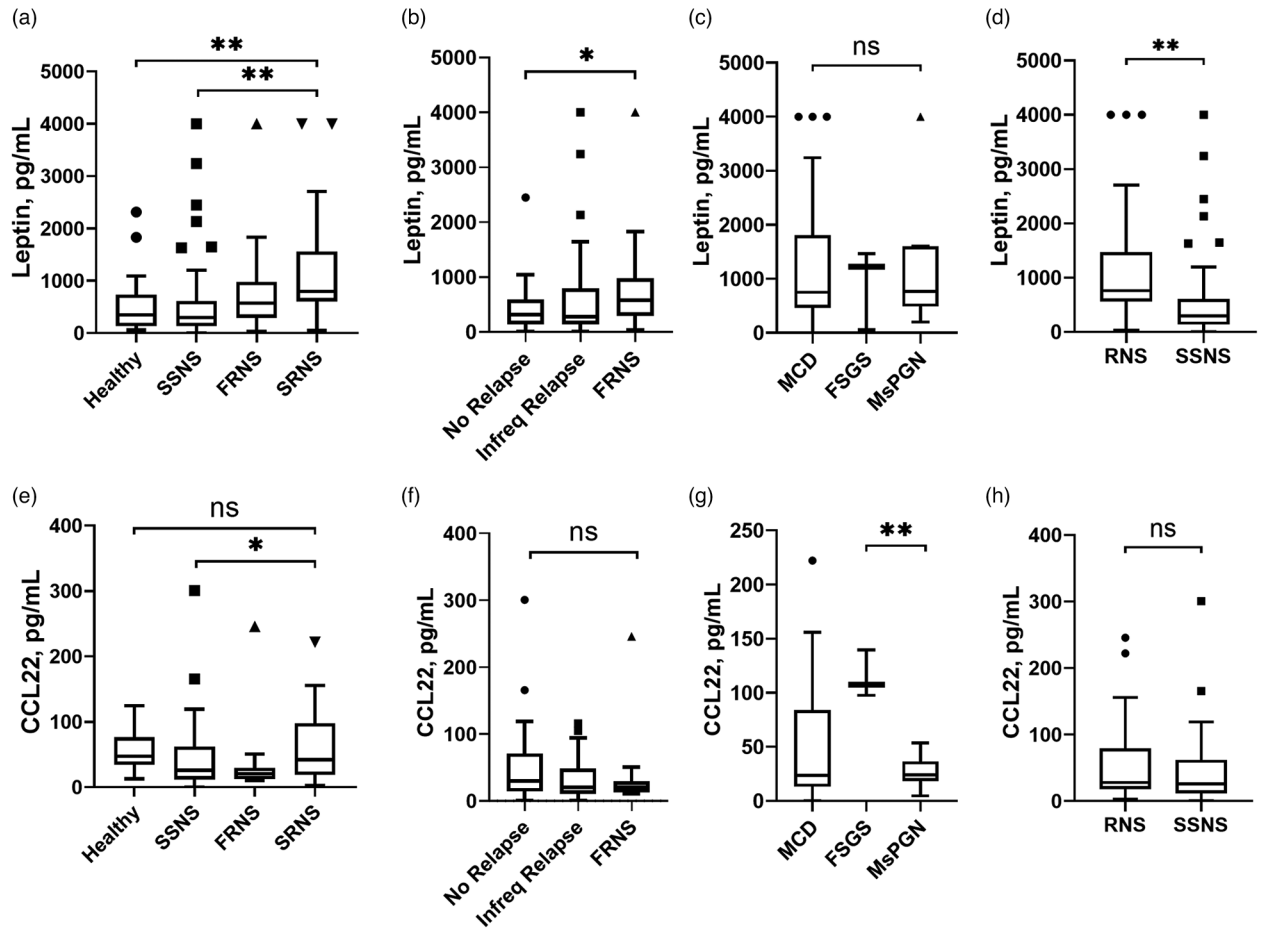

FIGURE 1
Serum concentration of Leptin and CCL22 in INS patients compared between different subgroups. INS, idiopathic nephrotic syndrome; SSNS, steroid-sensitive nephrotic syndrome; FRNS, frequent relapse nephrotic syndrome; SRNS, steroid-resistant nephrotic syndrome; RNS, refractory nephrotic syndrome; No Relapse, no recurred within 1 year after the first complete remission; Infreq Relapse, recurred once within 6 months or one to three times within 1 year after the first complete remission; MCD, minimal change disease; MsPGN, mesangial proliferative glomerulonephritis; FSGS, focal segmental glomerular sclerosis; *, statistically significant difference with $P < 0.05$; **, statistically significant difference with $P < 0.01$; ns, non-significant.

TABLE 3 Serum concentration of CCL22 and Leptin in INS patients with different steroid sensitivity.

Group	Cases	Leptin (pg/ml)	*P*-value to healthy	CCL22 (pg/ml)	*P*-value to healthy
Healthy	40	507.9 ± 479.8	/	55.9 ± 28.3	/
INS	117	697.2 ± 852.9	0.185	51.3 ± 84.2	0.737
SSNS	82	515.4 ± 676.9	0.950	40.6 ± 44.2	**0.048**
No relapse	42	406.5 ± 419.6	0.311	46.6 ± 53.2	0.333
Infreq relapse	40	629.8 ± 860.4	0.436	34.2 ± 31.6	**0.002**
RNS	35	1123.1 ± 1060.2	**0.001**	55.8 ± 62.0	0.994
FRNS	11	937.8 ± 1122.2	0.063	42.0 ± 68.5	0.312
SRNS	24	1208.1 ± 1044.1	**0.001**	62.4 ± 59.0	0.556

INS, idiopathic nephrotic syndrome; SSNS, steroid-sensitive nephrotic syndrome; FRNS, frequent relapse nephrotic syndrome; SRNS, steroid-resistant nephrotic syndrome; RNS, refractory nephrotic syndrome; No Relapse, no recurred within 1 year after the first complete remission; Infreq Relapse, recurred once within 6 months or one to three times within 1 year after the first complete remission; *P*-value: <0.05 means statistically significant difference.
The bold values mean $P < 0.05$ with the significant difference.

different pathological types, serum Leptin concentration was increased compared to healthy children, with a significant increase observed in MCD and MsPGN (Figure 1C). In patients with steroid resistance, there were minimal differences in Leptin concentration among the three pathological types (Table 2). Compared with group SSNS, Leptin was significantly higher in RNS group (Figure 1D). All data were listed in Table 2 and Table 3.

CCL22 decreased in SSNS

The concentration of serum CCL22 was significantly decreased in SSNS compared with SRNS, and no significant differences with healthy group (Figure 1E). The recurrence of INS patients was not related to CCL22 concentration (Figure 1F). CCL22 differed significantly among the three pathological types, with elevated levels in MCD and FSGS, and reduced levels in MsPGN (Figure 1G). Compared between RNS and SSNS, there were no significant differences in CCL22 concentration (Figure 1H). All data were listed in Table 2 and Table 3.

CCL22 and Leptin levels were elevated in INS patients with steroid resistance

Compared to SSNS group, the concentration of serum CCL22 and Leptin significantly increased in SRNS ($P = 0.046$ and $P = 0.007$). The diagnostic performance of serum CCL22 and Leptin in predicting RNS or SRNS was evaluated using the ROC curve shown in Figure 2. For SRNS prediction, the AUC of Leptin, CCL22, and the joint prediction index were 0.764, 0.640, and 0.806, respectively (Figure 2A). The cut-off of Leptin was 602.6 pg/ml, with positive predictive value (PPV) = 0.43, negative predictive value (NPV) = 0.93, The cut-off of CCL22 was 23.7 pg/ml, with PPV = 0.27, NPV = 0.87. For RNS prediction, the AUC of Leptin, CCL22, and the joint prediction index were 0.748, 0.578, and 0.756, respectively (Figure 2B). The cut-off of

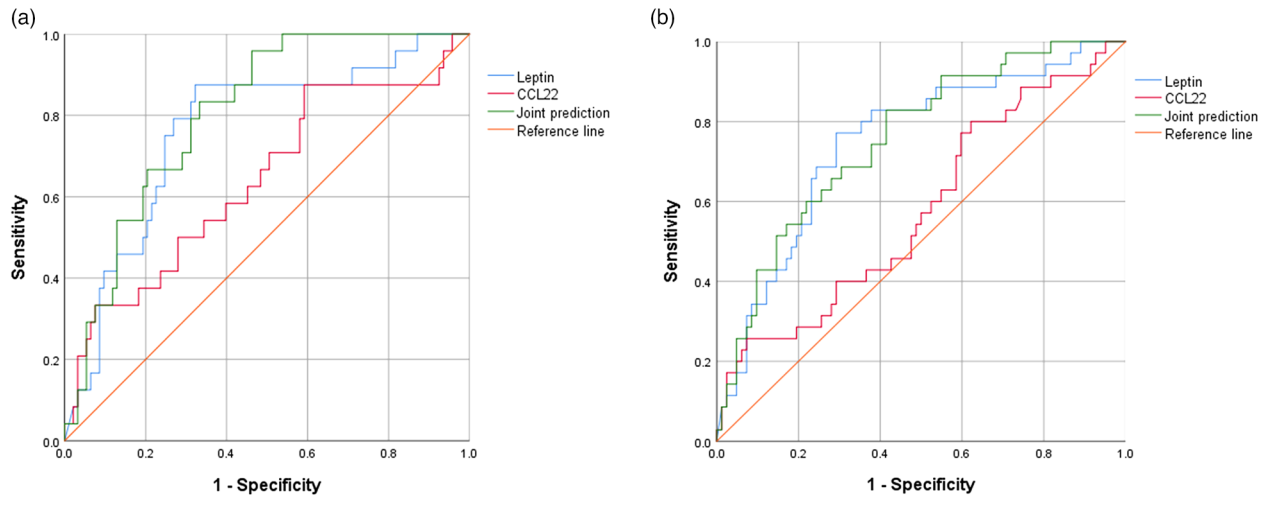

FIGURE 2
ROC curves of serum CCL22 and Leptin to predict SRNS or RNS. (A) For SRNS prediction. (B) For RNS prediction. Joint prediction: Joint diagnostic performance prediction by CCL22 and Leptin.

Leptin was 555.6 pg/ml, with PPV = 0.53, NPV = 0.88, The cut-off of CCL22 was 18.3 pg/ml, with PPV = 0.36, NPV = 0.80.

Discussion

The present study focused on the serum concentrations of CCL22 and Leptin in patients with idiopathic nephrotic syndrome. By prospectively collecting samples and conducting pre-steroid tests, we observed differences in CCL22 and Leptin concentrations among groups of patients with varying steroid responses. This study demonstrated the potential of CCL22 and Leptin to predict steroid resistance in the early stages of nephrotic syndrome.

Tregs play a crucial role in immune tolerance and are recognized as regulators of inflammation in INS. Previous research has shown that patients with steroid-resistant nephrotic syndrome exhibit lower levels of Tregs compared to those with steroid sensitivity (10). A slower increase in Tregs counts from disease onset to remission has been associated with a higher frequency of INS relapses (11). Severe disorders in lymphocyte subsets and abnormal regulation are believed to be involved in SRNS (12). Lymphocyte subsets, especially Tregs, may influence the treatment and prognosis of corticosteroids in INS.

Measuring Leptin level is a hot topic now, and more topics are focusing on its measurement as early childhood obesity, early childhood developmental assessment scores (13, 14). Our study provides confirmation that elevated Leptin levels are associated with steroid resistance in INS. It can be hypothesized that increased Leptin may diminish the response of kidney disease to steroid therapy by suppressing Treg levels, which aligns with findings from other studies in SRNS patients. The relationship between Leptin and steroid sensitivity has also been reported. Henmi K reported a significant decrease in PBMC response to prednisolone in INS patients and postulated that the Leptin receptor (OB-R) plays a crucial role (15). Increased serum Leptin has also been identified as a negative prognostic factor for the response to steroid therapy in autoimmune hepatitis (16).

CCL22 is an important factor in facilitating the migration of Tregs both *in vitro* and *in vivo* (17). In our study, there were significant differences between SRNS and SSNS patients in CCL22 concentrations, and were observed among patients with different pathological types. CCL22 levels were significantly elevated in the MCD and FSGS pathological types. We can infer that CCL22 may be related to the pathological changes in the glomerular structure. But more research data was needed to prove this.

As a small-sample, single-center observational study, our research has certain limitations. Expanding the sample size and conducting multi-center studies would provide more compelling conclusions. Furthermore, further investigations into the mechanisms of other related molecules would enhance the credibility of our findings and pave the way for subsequent explorations of the underlying mechanisms.

In conclusion, this study has demonstrated an association between serum concentrations of CCL22 and Leptin, measured prior to steroid therapy, and steroid resistance in childhood idiopathic nephrotic syndrome. Additionally, there were notable variations in CCL22 concentration across different glomerular pathological types, highlighting the need for further investigation into the molecular mechanisms involved.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://figshare.com/, 10.6084/m9.figshare.23704074.

Ethics statement

The studies involving humans were approved by Ethics Committee of Children's Hospital Zhejiang University School of Medicine (2021-IEC-037). The studies were conducted in accordance with the local legislation and institutional requirements. The human samples used in this study were acquired from From the remaining samples after clinical testing. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements.

Author contributions

ZP: Data curation, Formal Analysis, Funding acquisition, Project administration, Software, Writing – original draft. WL: Data curation, Methodology, Software, Writing – original draft. YJ: Data curation, Investigation, Software, Writing – original draft. WX: Data curation, Investigation, Methodology, Resources, Writing – original draft. HF: Conceptualization, Supervision, Validation, Visualization, Writing – review & editing. JM: Supervision, Validation, Visualization, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Remission and long-term remission of pediatric-onset systemic lupus erythematosus

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Objectives: Systemic lupus erythematosus (SLE) is a chronic autoimmune disease with diverse clinical presentations and prognoses. Remission can be achieved with or without glucocorticoid (GC) use, and several recent studies have suggested that long-term remission can be achieved in a small portion of patients. Nevertheless, few studies have investigated remission or long-term remission in the pediatric-onset SLE subgroup. This study analyzed the characteristics and factors associated with long-term remission and GC use in pediatric-onset SLE.
Methods: We enrolled 226 patients aged <18 years who received a diagnosis of SLE between January 2006 and December 2016. Three remission condition groups were defined: (A) complete remission, (B) clinical remission off GCs, and (C) clinical remission on GCs. Long-term remission was defined as remission for more than 5 years. We analyzed the treatment durations before remission, durations of remission, and risk factors for non-remission with persistent GC use.
Results: During follow-up, 8 patients (3.5%) achieved complete remission, 35 patients (15.5%) achieved clinical remission off GCs, and 93 patients (41.2%) achieved clinical remission on GCs. In groups A, B, and C, 12.5%, 68.6%, and 65.6% of patients, respectively, remained in remission for >1 year.
Conclusion: This study assessed remission of pediatric-onset SLE. Up to 60.2% of patients had clinical remission after treatment, and 19% of patients achieved remission off GCs. Long-term remission is rarer in pediatric-onset SLE than in adult-onset SLE.

KEYWORDS

pediatrics, prognosis, remission, systemic lupus erythematosus, glucocorticoid

1. Introduction

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease. Although the prognosis and survival rate of patients with SLE have improved significantly, relapses and are still common (1). Approximately 10%–20% of patients with SLE have pediatric-onset SLE (2, 3). Pediatric-onset SLE is more active, more frequently involves the kidneys and neuropsychiatry, and causes damage earlier than adult-onset SLE. Immunosuppressive treatment is more common among patients with pediatric-onset SLE than among those with adult-onset SLE (2, 4–7). The prognoses, including long-term survival and renal outcomes, are worse among patients with pediatric-onset SLE than among those with adult-onset SLE (8–10).

Achieving remission is possible for patients with SLE. Studies have reported long-term remission in patients with SLE; however, these studies have provided varied definitions of

"long-term" or "prolonged" (1, 11–14). Only a small portion of patients with adult-onset SLE achieve long-term remission. Remission is even rarer among patients with pediatric-onset SLE than among those with adult-onset SLE. Achieving remission is one of the primary goals of SLE treatment. Remission can influence health-related quality of life and may protect patients against some of the subsequent morbidities of SLE, including flare-up events and cardiovascular and renal diseases (15–17). Though there were several works of literatures reporting the remission of SLE adult patients, few reported the rates or characteristics of remission for pediatric-onset SLE.

This study aimed to clarify the characteristics of remission for pediatric-onset SLE. We followed the definition of SLE remission from the literature of Zen et al. (13), which considers clinical conditions, serological activities, and treatments. We analyzed the remission of pediatric-onset SLE, including its rate, duration, and predictors.

2. Materials and methods

2.1. Patients

We used data from the Taichung Veterans General Hospital Research Database, which is managed by the Clinical Informatics Research & Development Center of Taichung Veterans General Hospital. Patient information was anonymized before being made available for our research. The research protocol was approved by the Institutional Review Board of Taichung Veterans General Hospital (IRB number: CE20204A).

Patients who were admitted to our wards or visited our outpatient clinic and who received a diagnosis of SLE between January 2006 and December 2016 were included. Patients who fulfilled the 1997 ACR (18) or 2012 SLICC (19) classification criteria were defined as having SLE. Patients aged >18 years were excluded. Laboratory data were retrospectively analyzed. Treatments included glucocorticoid (GC), high-dose GC, antimalarial, immunosuppressive, and biological drug therapies.

A total of 329 patients were initially screened. We excluded patients with less than three visits to the outpatient clinic, those with follow-up periods <6 months, those who did not receive a GC prescription, and those who were lost to follow-up in our hospital. A total of 226 patients were subsequently included in the analysis. We collected their serological and laboratory data (including complete blood count, differential count, urine analysis, and biochemistry function) and their data on clinical disease activity and complications.

2.2. Disease activity and definitions of remission

Three definitions of remission were considered according to the disease activity and treatment: (A) complete remission, (B) clinical remission off GCs, and (C) clinical remission on GCs (**Table 1**). GCs are the mainstay of treatment in pediatric patients with SLE (8).

(A) Complete remission: no clinical or serological disease activity; GC-free and immunosuppressant-free; and antimalarials were allowed.

(B) Clinical remission off GC: serologically active, clinically quiescent disease; GC-free and immunosuppressant-free; and antimalarials were allowed.

(C) Clinical remission on GC: serologically active, clinically quiescent disease; immunosuppressant-free; GCs and antimalarials were allowed; and long-term remission was defined as remission for more than 5 years.

2.3. Clinical conditions and laboratory data

Clinical conditions were judged based on laboratory data and diagnoses in inpatient and outpatient medical records. Laboratory data included complete blood count, renal function, liver function, C-reactive protein, erythrocyte sedimentation rate, and urine analysis results. Serological data included the titers of antinuclear antibodies and anti–double stranded DNA (anti-dsDNA) antibodies; Anti-dsDNA would be considered positive if it is positive in anytime during the disease course. The highest and lowest C3 and C4 indicated the highest and lowest level in anytime during the disease course. Treatments included nonsteroidal anti-inflammatory drugs, GCs, disease-modifying antirheumatic drugs, and immunosuppressives and biological therapies. Antimalarials were allowed in all remission groups.

2.4. Statistical analysis

Analyses were performed using Statistical Package for the Social Sciences (IBM SPSS version 22.0; New York, USA). Normal distribution of the data was verified with the Kolmogorov–Smirnov test. Continuous data are expressed as median and interquartile range. Continuous data were compared using the Kruskal–Wallis test and Mann–Whitney U test. Categorical data are expressed as numbers and percentages. Categorical data were compared using the chi-square and Fisher's

TABLE 1 Definitions of remission according to clinical, serological and therapeutic status.

	Disease activity		Treatment		
	Clinical	Serology	Antimalarials	Steroid	Immunosuppressants
Complete remission	—	—	±	—	—
Clinical remission off steroids	—	±	±	—	—
Clinical remission on steroids	—	±	±	+	—

exact tests. Statistical significance was indicated by p values of <0.05. Cox regression analysis was performed to identify baseline patient characteristics (including laboratory data and clinical conditions) associated with the time to remission, with adjustment for baseline disease activity and treatment. We examined one variable at a time with Cox regression, including demographic and immunological characteristics and specific types of disease activity. Patient characteristics, especially the initial and lowest C3/C4 levels, were analyzed, and the results are expressed as hazard ratios and 95% confidence intervals.

3. Results

3.1. Patient characteristics

Among the 226 patients, 8 (3.5%) achieved complete remission, 35 (15.5%) achieved clinical remission off GCs, and 93 (41.2%) achieved clinical remission on GCs. In total, 90 patients (39.8%) did not achieve any level of remission (**Table 2**). Characteristics of these four groups are summarized in **Table 2**, showing the mean age at the time of diagnosis, gender distribution, serological data (antinuclear antibodies and anti-dsDNA antibodies), and clinical presentations. The mean age at initial diagnosis was 13.9 ± 3.1 years. The male-to-female ratio was 1:7.4. The three clinical remission groups exhibited differences in the lowest antinuclear antibodies titers ($p = 0.008$), the highest C3 ($p < 0.001$) and highest C4 ($p = 0.001$) levels, the percentages of ever positive anti-dsDNA antibodies ($p = 0.038$), leukopenia ($p = 0.002$), hematuria ($p = 0.034$), and seizure ($p = 0.002$).

3.2. Treatment duration before remission

In the complete remission group, the treatment duration before remission was 899 ± 668.8 days. In the clinical remission off GCs group, this duration was 840.9 ± 708.7 days. By contrast, 72 patients (77.4%) achieved clinical remission on GCs within 1 year, with a mean duration of treatment of 312 ± 609.7 days. The difference in the treatment duration before remission between the clinical remission on GCs group and the other groups was statistically significant ($p < 0.001$).

3.3. Duration of remission

The duration of remission of the three remission groups is provided in **Figure 1**. The average duration of complete remission was 248.1 ± 141.43 days. Only one patient (12.5%) remained in complete remission for more than 1 year (1.29 years). In the clinical remission off GCs group, 24 patients (68.6%) remained in remission for more than 1 year. In the clinical remission on GCs group, 61 patients (65.6%) remained in remission for periods exceeding 1 year; 30 patients (32.3%) remained in remission for more than 5 years (long-term remission).

3.4. Characteristics of patients who achieved complete remission

Compared with patients who did not achieve complete remission, the eight patients who achieved complete remission had lower initial antinuclear antibody titers ($p = 0.043$) and the lowest antinuclear antibody titers ($p = 0.004$). Only one patient (12.5%) who achieved complete remission had positive anti-dsDNA antibodies. By contrast, among the 218 patients who did not achieve complete remission, 129 (59.2%) had positive anti-dsDNA antibodies ($p = 0.011$). No significant differences were observed in C3 and C4 levels between these two groups. The complete remission group exhibited a lower rate of leukopenia (12.5% vs. 58.3%, $p = 0.023$) but a higher rate of seizure (25% vs. 2.3%, $p = 0.021$). Furthermore, no significant differences were observed in the rates of cardiovascular accident, headache, organic brain syndrome, psychosis, visual disturbance, and vasculitis.

3.5. Analyses of patients who achieved clinical remission

We further divided patients who achieved clinical remission into two subgroups, off GC and on GC, as mentioned earlier in this article. One subgroup discontinued GCs for a period, and the other subgroup used GCs throughout the study period. The characteristics of these two subgroups are shown in **Table 3**. No significant differences in patient characteristics, namely serological findings, complement C3/C4 levels, laboratory data, or severe clinical manifestations, were observed between these two subgroups. However, in Cox regression, we observed that when the initial C3, initial C4, and lowest C4 levels were increased by 1, the probability of discontinuing GCs was increased by 1.01-fold, 1.07-fold, and 1.07-fold respectively after age and gender adjustment (**Table 4**).

4. Discussion

Few studies have investigated remission among patients with pediatric-onset SLE. Studies have mainly focused on renal involvement (9, 10). In the present study, 60.2% of patients achieved some level of remission.

The remission rate varies among studies involving patients 18 years or older. In a review in 2019 (12), Ruiz-Irastorza et al. reported that the 1-year remission rate ranged from 9.2% to 54.6%, regardless of treatment. However, no strict or universal definitions of remission or long-term remission were applied; therefore, the results may not be comparable between different studies. The definition of the remission of SLE was revised in 2021 by an international task force known as the DORIS Task Force (16). The definition included (1) clinical SLE disease activity index = 0 (2), Physician Global Assessment score <0.5 (on a scale of 0–3) (3), prednisolone dose equal to or less than 5 mg/day, and (4) stable use of antimalarials,

TABLE 2 Patient demographics and clinical characteristics overall and according to different types of remission.

	No-remission ($n = 90$)		Complete remission ($n = 8$)		Clinical remission off steroids ($n = 35$)		Clinical remission on steroids ($n = 93$)		Overall ($n = 226$)		p value
Age of SLE diagnosis	14.0	±3.1	13.5	±2.5	14.3	±2.7	13.6	±3.3	13.9	±3.1	0.605
Gender (male:female)	9: 81		0: 8		5: 30		13: 80		27: 199		0.363
Initial ANA titer											0.151
1:40	0	(0.0%)	0	(0.0%)	1	(2.9%)	3	(3.2%)	4	(1.8%)	
1:80	5	(5.6%)	3	(37.5%)	1	(2.9%)	9	(9.7%)	18	(8.0%)	
1:160	17	(18.9%)	3	(37.5%)	3	(8.6%)	16	(17.2%)	39	(17.3%)	
1:320	18	(20.0%)	1	(12.5%)	6	(17.1%)	15	(16.1%)	40	(17.7%)	
1:640	13	(14.4%)	0	(0.0%)	9	(25.7%)	17	(18.3%)	39	(17.3%)	
1:1,280	21	(23.3%)	1	(12.5%)	7	(20.0%)	15	(16.1%)	44	(19.5%)	
1:2,560	16	(17.8%)	0	(0.0%)	8	(22.9%)	16	(17.2%)	40	(17.7%)	
1:5,120	0	(0.0%)	0	(0.0%)	0	(0.0%)	2	(2.2%)	2	(0.9%)	
Lowest ANA titer											0.008**
1:40	4	(4.4%)	3	(37.5%)	5	(14.3%)	12	(12.9%)	24	(10.6%)	
1:80	12	(13.3%)	5	(62.5%)	5	(14.3%)	18	(19.4%)	40	(17.7%)	
1:160	15	(16.7%)	0	(0.0%)	5	(14.3%)	16	(17.2%)	36	(15.9%)	
1:320	14	(15.6%)	0	(0.0%)	8	(22.9%)	16	(17.2%)	38	(16.8%)	
1:640	14	(15.6%)	0	(0.0%)	8	(22.9%)	11	(11.8%)	33	(14.6%)	
1:1,280	19	(21.1%)	0	(0.0%)	4	(11.4%)	9	(9.7%)	32	(14.2%)	
1:2,560	12	(13.3%)	0	(0.0%)	0	(0.0%)	10	(10.8%)	22	(9.7%)	
1:5,120	0	(0.0%)	0	(0.0%)	0	(0.0%)	1	(1.1%)	1	(0.4%)	
Highest ANA titer											0.210
1:80	1	(1.1%)	0	(0.0%)	1	(2.9%)	1	(1.1%)	3	(1.3%)	
1:160	13	(14.4%)	2	(25.0%)	2	(5.7%)	16	(17.2%)	33	(14.6%)	
1:320	24	(26.7%)	4	(50.0%)	4	(11.4%)	15	(16.1%)	47	(20.8%)	
1:640	11	(12.2%)	1	(12.5%)	6	(17.1%)	19	(20.4%)	37	(16.4%)	
1:1,280	23	(25.6%)	1	(12.5%)	10	(28.6%)	15	(16.1%)	49	(21.7%)	
1:2,560	18	(20.0%)	0	(0.0%)	11	(31.4%)	24	(25.8%)	53	(23.5%)	
1:5,120	0	(0.0%)	0	(0.0%)	1	(2.9%)	3	(3.2%)	4	(1.8%)	
Anti-dsDNA antibodies (positive %)	57	(63.3%)	1	(12.5%)	18	(51.4%)	54	(58.1%)	130	(57.5%)	0.038*
Initial C3 (mg/dl)	56.1	±43.7	62.3	±55.7	58.8	±52.6	52.3	±47.4	55.2	±47.0	0.662
Lowest C3 (mg/dl)	46.4	±40.6	39.3	±41.0	37.1	±36.7	45.6	±42.0	44.3	±40.5	0.902
Highest C3 (mg/dl)	97.3	±34.8	112.8	±15.4	125.5	±25.9	111.1	±37.8	108.2	±35.6	<0.001**
Initial C4 (mg/dl)	8.5	±8.7	12.8	±11.2	9.5	±10.8	9.6	±9.3	9.3	±9.4	0.793
Lowest C4 (mg/dl)	6.5	±8.2	8.9	±8.9	5.5	±7.7	6.7	±7.9	6.5	±8.0	0.492
Highest C4 (mg/dl)	20.5	±12.0	24.0	±9.3	30.6	±13.0	24.8	±11.2	24.0	±12.2	0.001**
Leukopenia [number(%)]	44	(48.9%)	1	(12.5%)	27	(77.1%)	56	(60.2%)	128	(56.6%)	0.002**
Thrombocytopenia [number(%)]	38	(42.2%)	1	(12.5%)	21	(60.0%)	40	(43.0%)	100	(44.2%)	0.072
Hematuria [number(%)]	46	(51.1%)	6	(75.0%)	26	(74.3%)	63	(67.7%)	141	(62.4%)	0.034*
Pyuria [number(%)]	64	(71.1%)	7	(87.5%)	30	(85.7%)	69	(74.2%)	170	(75.2%)	0.310
Proteiuria [number(%)]	22	(24.4%)	2	(25.0%)	9	(25.7%)	23	(24.7%)	56	(24.8%)	0.999
CVA [number(%)]	0	(0.0%)	0	(0.0%)	2	(5.7%)	1	(1.1%)	3	(1.3%)	0.089
Headache [number(%)]	7	(7.8%)	1	(12.5%)	1	(2.9%)	12	(12.9%)	21	(9.3%)	0.321
Organic brain syndrome [number(%)]	0	(0.0%)	0	(0.0%)	0	(0.0%)	1	(1.1%)	1	(0.4%)	0.697
Psychosis [number(%)]	1	(1.1%)	0	(0.0%)	2	(5.7%)	0	(0.0%)	3	(1.3%)	0.088
Seizure [number(%)]	1	(1.1%)	2	(25.0%)	2	(5.7%)	2	(2.2%)	7	(3.1%)	0.002**
Visual disturbance [number(%)]	0	(0.0%)	0	(0.0%)	0	(0.0%)	1	(1.1%)	1	(0.4%)	0.697
Vasculitis [number(%)]	2	(2.2%)	0	(0.0%)	3	(8.6%)	5	(5.4%)	10	(4.4%)	0.388

Chi-Square test. Kruskal Wallis test.
Continuous data were expressed median and IQR.
Categorical data were expressed number and percentage.
*$p < 0.05$.
**$p < 0.01$.

immunosuppressives, and biologics. Serology was not included in the definition. Whether this definition can be applied to pediatric-onset SLE requires further validation.

In the further analysis of the characteristics of patients who achieved complete remission, our study revealed that the complete remission group had lower initial antinuclear antibody

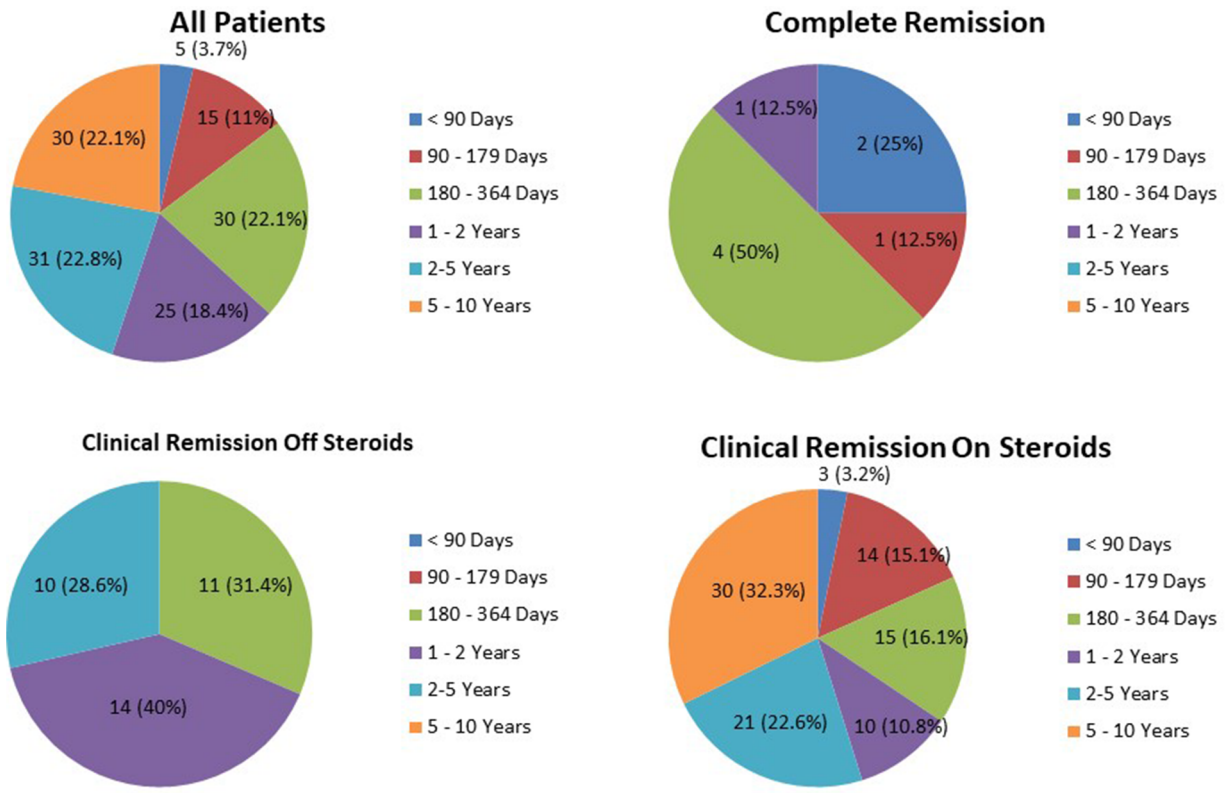

FIGURE 1
The duration of remission of the three remission groups.

titers, fewer positive anti-dsDNA antibodies, and a lower rate of leucopenia. Although statistically significant, our relatively small sample size may lead to different results compared with other studies. ANA titer does not reflect disease activity. However, in some studies, ANA titers were used to predict the highest cumulative disease activity and immunosuppressants/ biologics use (20).

Wilhelm et al. (14) observed that both baseline positive anti-dsDNA and baseline low C4 levels are negative predictors of complete remission with and without treatment. In our study, we found that in patients with pediatric-onset SLE, initial complement C3 and C4 levels were associated with the discontinuation of GCs, which is similar to the findings of Wilhelm et al. When the initial C3, initial C4, and lowest C4 levels were increased by 1, the probability of discontinuing GCs was increased by 1.01-fold, 1.07-fold, and 1.07-fold respectively after age and gender adjustment in this study.

Long-term remission is not common, even in adult-onset SLE. A recent review suggested that among patients with SLE, approximately 15% can achieve prolonged remission, 70% have a relapse-remitting disease course, and the remaining 15% have persistently active disease (21). Zen et al. (13) found that 37% of patients achieved prolonged (more than 5 years) remission, 7.1% achieved prolonged complete remission, 14.7% achieved prolonged clinical remission off GCs, and 15.6% achieved prolonged clinical remission on GCs. In an observational study including two cohorts with similar SLE manifestations at diagnosis (12), Ruiz-Irastorza et al. reported a comparison of remission rates between two different treatment strategies. Patients recruited in the longitudinal Cruces Lupus Cohort, with more frequently treated with hydroxychloroquine, methotrexate, and pulse methylprednisolone, were more likely to have prolonged (4 years) clinical remission than the Bordeaux Lupus Cohort (70% and 28% respectively). In a retrospective cohort study involving 2,121 patients that was conducted by Jakez-Ocampo et al. (11), only 44 patients achieved sustained remission for more than 10 years. Tselios et al. described an atypical monophasic course (22). They also found that 10.1% of patients with SLE achieved prolonged clinical remission for more than 10 years, and that 20 patients (7.5%) achieved remission for the entire follow-up, averaging 18 years.

Various studies have reported different findings for baseline (first visit) clinical or immunological variables between patients with SLE who did and did not achieve prolonged remission. Tselios et al. (22) reported no clinical or immunological differences between patients with SLE who did and did not achieve prolonged remission. In a cohort study, Jakez-Ocampo et al. (11) found that older age at disease onset (with a p value of 0.055) was associated with prolonged remission (more than 10 years). This finding may partially explain why prolonged remission was rare in our study, which focused on patients with pediatric-onset SLE.

TABLE 3 Patient demographics and cumulative disease manifestations according to GC use.

	On steroid remission ($n = 93$)		Off steroid remission ($n = 43$)		p value
Age of SLE diagnosis	13.6 ± 3.3		14.1 ± 2.7		0.461
Gender (male: female)	13: 80		5: 38		0.917
Initial ANA titer					0.984
1:40	3	(3.2%)	1	(2.3%)	
1:80	9	(9.7%)	4	(9.3%)	
1:160	16	(17.2%)	6	(14.0%)	
1:320	15	(16.1%)	7	(16.3%)	
1:640	17	(18.3%)	9	(20.9%)	
1:1,280	15	(16.1%)	8	(18.6%)	
1:2,560	16	(17.2%)	8	(18.6%)	
1:5,120	2	(2.2%)	0	(0.0%)	
Lowest ANA titer					0.374
1:40	12	(12.9%)	8	(18.6%)	
1:80	18	(19.4%)	10	(23.3%)	
1:160	16	(17.2%)	5	(11.6%)	
1:320	16	(17.2%)	8	(18.6%)	
1:640	11	(11.8%)	8	(18.6%)	
1:1,280	9	(9.7%)	4	(9.3%)	
1:2,560	10	(10.8%)	0	(0.0%)	
1:5,120	1	(1.1%)	0	(0.0%)	
Highest ANA titer					0.759
1:80	1	(1.1%)	1	(2.3%)	
1:160	16	(17.2%)	4	(9.3%)	
1:320	15	(16.1%)	8	(18.6%)	
1:640	19	(20.4%)	7	(16.3%)	
1:1,280	15	(16.1%)	11	(25.6%)	
1:2,560	24	(25.8%)	11	(25.6%)	
1:5,120	3	(3.2%)	1	(2.3%)	
Anti-dsDNA antibodies	54	(58.1%)	19	(44.2%)	0.185
Initial C3 ($n = 253$)	52.3	±47.4	59.5	±52.5	0.243
Lowest C3 ($n = 253$)	45.6	±42.0	37.6	±37.0	0.827
Highest C3 ($n = 253$)	111.1	±37.8	123.0	±24.6	0.237
Initial C4 ($n = 265$)	9.6	±9.3	10.2	±10.8	0.852
Lowest C4 ($n = 265$)	6.7	±7.9	6.2	±8.0	0.513
Highest C4 ($n = 265$)	24.8	±11.2	29.3	±12.6	0.074
Leukopenia [number(%)]	56	(60.2%)	28	(65.1%)	0.721
Thrombocytopenia [number(%)]	40	(43.0%)	22	(51.2%)	0.482
Hematuria [number(%)]	63	(67.7%)	32	(74.4%)	0.557
Pyuria [number(%)]	69	(74.2%)	37	(86.0%)	0.184
Proteinuria [number(%)]	23	(24.7%)	11	(25.6%)	1.000
CVA [number(%)]	1	(1.1%)	2	(4.7%)	0.235
Headache [number(%)]	12	(12.9%)	2	(4.7%)	0.224
Organic brain syndrome [number(%)]	1	(1.1%)	0	(0.0%)	1.000
Psychosis [number(%)]	0	(0.0%)	2	(4.7%)	0.098
Seizure [number(%)]	2	(2.2%)	4	(9.3%)	0.079
Visual disturbance [number(%)]	1	(1.1%)	0	(0.0%)	1.000
Vasculitis [number(%)]	5	(5.4%)	3	(7.0%)	0.708

TABLE 4 Variables independently associated with remission off GCs based on Cox regression controlling for disease activity and treatments.

	Univariate			Adjusted for age and gender		
	HR	(95% CI)	p value	HR	95% CI	p value
Initial C3	1.01	(1.003–1.02)	0.003**	1.01	(1.004–1.02)	0.002**
Initial C4	1.06	(1.02–1.09)	0.001**	1.07	(1.03–1.11)	0.001**
Lowest C4	1.06	(1.02–1.11)	0.007**	1.07	(1.02–1.12)	0.004**

Cox regression.
**$p < 0.01$.

Biological agents, such as rituximab and belimumab, are emerging as new treatment choices for SLE (21). Both rituximab and belimumab demonstrated favorable safety and efficacy for treating pediatric-onset SLE (23, 24). Other therapies, such as anti-interferon monoclonal antibodies and Janus kinase inhibitors, are being investigated for treating pediatric-onset SLE (25). Whether biologics can affect remission and long-term remission rates in both pediatric-onset and adult-onset SLE is unclear.

The present study has several limitations. This was a retrospective, single-center cohort study. The number of included patients was relatively small, leading to possible insufficient statistical power for some of our results. All of our patients were Asian/Taiwanese; thus, our findings may not be generalizable to other ethnic groups. The definition of remission used in our study may differ from that used in other studies; thus, the results may not be comparable with those of other studies; for example, in our study, immunosuppressants were not allowed for any degree of remission, whereas they were allowed in other studies and according to the 2021 definition established by the DORIS Task Force. The status of lupus nephritis and renal remission, occurring more often and more severely among patients with pediatric-onset SLE than among those with adult-onset SLE (9, 26), was not included in this study due to poor correlations observed between systemic and renal activities (9). We checked the patient's ANA titers every 6–12 months in follow-ups, but not strictly; this may affect our interpretation of the lowest ANA titer in the study.

In conclusion, our study showed that more than 60% of patients with pediatric-onset SLE achieved some degree of remission, but only a small portion achieved long-term remission. Further observational studies focusing on pediatric-onset SLE are necessary for a more accurate prediction of clinical outcomes in this patient group.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by Institutional Review Board of Taichung Veterans General Hospital (IRB number: CE20204A). The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements.

Author contributions

YH: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Supervision, Validation, Writing – original draft, Writing – review & editing. YC: Conceptualization, Methodology, Validation, Writing – original draft, Writing – review & editing. CH: Data curation, Formal Analysis, Investigation, Methodology, Validation, Writing – review & editing. MT: Conceptualization, Formal Analysis, Investigation, Validation, Writing – review & editing. LF: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Resources, Validation, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Global burden of chronic kidney disease in adolescents and young adults, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019

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Background: The global status of chronic kidney disease (CKD) is underestimated, particularly the burden on adolescents and young adults (early-onset, aged 15–39).

Objective: We aim to investigate the pattern and trend of early-onset CKD from 1990 to 2019.

Methods: We analyzed age-specific rates of early-onset CKD incidence, death, and disability-adjusted life years (DALY) using Global Burden of Disease Study 2019 data. We examined the global, regional, national, gender-based, age group-based, and temporal changes of early-onset CKD burden from 1990 to 2019, as well as proportional DALY attributions of various risk factors.

Results: From 1990 to 2019, the global age-specific incidence rate (per 100,000 population) significantly increased from 25.04 (95% confidence interval 18.51, 31.65) to 32.21 (23.73, 40.81) for early-onset CKD. However, the global age-specific death rate significantly decreased from 2.96 (2.76, 3.15) to 2.86 (2.61, 3.11), and the age-specific DALY rate remained stable. Regarding sociodemographic indexes (SDI), countries with middle SDI had the highest incidence rates and the fastest increasing trends, while those with low and low-middle SDI experienced the highest death and DALY rates. Women had a generally higher age-specific incidence rate than men, whereas men showed higher age-specific death and DALY rates. In addition, the burdens of CKD increased with age among adolescents and young adults. Moreover, the main attributable risk factors for DALY of early-onset CKD were high systolic blood pressure (SBP), fasting plasma glucose (FPG), and body mass index (BMI).

Conclusion: The age-specific incidence rate of early-onset CKD increased significantly from 1990 to 2019, and the age-specific DALY rate remained stable. High SBP, high FPG, and high BMI were the primary risk factors. Targeted prevention and healthcare measures should be developed considering age, gender, and region.

KEYWORDS

chronic kidney disease, early-onset, adolescents and young adults, GBD 2019, global burden

Introduction

Chronic kidney disease (CKD) is a pressing global health concern and a significant independent risk factor for cardiovascular disease, with over 650 million individuals affected by CKD in 2017 (1). CKD exhibits diverse etiologies and can manifest across all age groups, each influenced by distinct risk factors and disease profiles. Notably, the causes of death differ among middle-aged and elderly individuals with CKD (eg. renal failure), compared to adolescents and young adults with early-onset CKD (eg. cardiovascular causes and infections) (2).

Although less than 5% of adolescents with CKD progress to end-stage kidney disease, they still face a much higher death rate than that of healthy peers (3). Moreover, it has been demonstrated that the CKD burden appears to be increasing among young people (4), indicating that an up-to-date understanding of the global burden of early-onset CKD is essential for its primary intervention, secondary intervention, and health care. Adolescents and young adults grappling with CKD necessitate tailored healthcare interventions due to their unique set of complications, including hypertension, volume overload, metabolic bone disorders, and various physiological irregularities (5). Their clinical management often involves a complex array of interventions, ranging from polypharmacy for addressing bone disorders to specialized procedures like enteral feeding and catheterizations (6). Enhancing self-management skills and health literacy among this demographic is paramount, as limited health literacy is linked to unfavorable treatment adherence and outcomes (7).

Hence, a comprehensive understanding of the worldwide impact of early-onset CKD is imperative to allocate healthcare resources optimally across diverse regions. Despite existing reports on CKD deaths and disability-adjusted life years (DALY) between 1990 and 2017 (1), there remains a dearth of research specifically investigating the global burden of early-onset CKD (aged 15–39). Our research examines trends in age-specific incidence, death, and DALY rates of early-onset CKD, along with identifying attributable risk factors, across various demographic and sociodemographic categories from 1990 to 2019. This holistic analysis is essential for developing targeted primary and secondary interventions to achieve the United Nations Sustainable Development Goal of reducing premature mortality from non-communicable diseases by a third by 2030 (8).

Abbreviations: CKD, chronic kidney disease; GBD 2019, Global Burden of Disease Study 2019; DALY, disability-adjusted life years; SDI, socio-demographic index; EAPC, estimated annual percentage change; BMI, high body-mass index; FPG, fasting plasma glucose; SBP, systolic blood pressure.

Methods

Data sources

GBD 2019 project estimated the incidence, death, and DALY for 369 diseases and injuries across 204 countries and territories from 1990 to 2019. An overview of GBD 2019 data collection, modeling, and analysis was provided in the Supplementary Materials (Appendix 1). Data of cause-specific incidence rate, death rate, DALY rate, and DALY attributable to different risk factors (with corresponding 95% confidence intervals [CI]) of CKD, in adolescents and young adults from 1990 to 2019, by age, sex, year, region, and nations were obtained using the Global Health Data Exchange GBD results tool (http://ghdx.healthdata.org/gbd-results-tool).

Relevant definitions

CKD was defined by elevated urinary albumin to creatinine ratio, decreased estimated glomerular filtration rate, or end-stage kidney disease (1). Our study specifically focused on early-onset CKD, characterized by an age of onset between 15 and 39 years. DALY is a comprehensive measure of disease burden, combining years of life lost due to premature death and years lived with disability. The sociodemographic index (SDI) serves as an indicator of a region's development status, encompassing factors such as fertility rates among females under 25, average years of education for individuals aged 15 and above, and lag-distributed income per capita. 204 countries and territories were categorized into quintiles based on SDI level: low, low-middle, middle, high-middle, and high.

Temporal trends were described using the estimated annual percentage change (EAPC) (9). Positive EAPC indicated an increasing incidence/death/DALY rate, while negative suggested a decline in the rates.

Statistical analysis

We evaluated the number and the age-specific rates of incidence, death, and DALY in 2019, and their EAPCs (95% CI) (1990–2019) associated with early-onset CKD. We quantified age-specific trends in CKD burden based on gender and age group with a 5-year interval. Differences between males and females were calculated using the age-specific rates in males minus that in females. Based on the GBD 2019 country-level data, we mapped the age-specific incidence rates, death rates, DALY rates, and their EAPCs for 204 countries and regions. Additionally, we depicted the relationship between the SDI and the incidence/death/DALY rate by GBD region using Gaussian process regression. Furthermore, Spearman's correlation coefficients between the SDI and EAPCs of the age-specific incidence/death/DALY rate were calculated. All statistical analyses and plots were implemented using the R program (Version 4.1.2). A two-tailed p-value < 0.05 was considered statistically significant.

Results

Global burden of early-onset CKD in 2019

In 2019, the global incidence, death, and DALY rates (per 100,000 population) for early-onset CKD were 32.21 (95% CI 23.73 to 40.81), 2.86 (2.61 to 3.11), and 236.85 (209.03 to 268.91), respectively. The global incidence and death rates were slightly higher in females (34.33) than in males (30.14), whereas the death and DALY rates were higher for men (3.32 and 253.82, respectively) than for women (2.38 and 219.45, respectively) (Table 1). Age-specific incidence, death, and DALY rates all increased with age groups (Supplementary Figure 1).

By SDI category, the highest incidence rate was in middle SDI countries (37.49, 27.3 to 47.6). Low and low-middle SDI countries had the highest death rate (3.78, 3.28 to 4.29) and DALY rate (290.52, 257.55 to 326.58), respectively (Table 1). The lowest incidence, death, and DALY rates were found in high SDI countries, with 22.97, 0.8, and 95.66 per 100, 000 population, respectively (Table 1). At the regional level, the greatest incidence rate of early-onset CKD was seen in parts of Central Asia, whereas parts of Central Latin America had the highest death and DALY rates (Table 1). At the country-level analysis, six nations (Palau, Micronesia, Kiribati, Nauru, El Salvador, and Solomon Islands) demonstrated consistent presence within the top 10 ranks for incidence, death, and DALY rates in 2019 (Figure 1; Supplementary Tables 1–3).

The differences in age distribution in incidence, death, and DALY rates between men and women were analyzed by age groups and SDI categories. Globally, the differences in incidence, death, and DALY

TABLE 1 Age-specific incidence, death, and DALY rates (per 100,000 persons) of the early-onset CKD in adolescents and young adults, along with their EAPCs globally, categorized by sex, SDI categories, and GBD regions from 1990 to 2019.

Location name	Incidence rate			Death rate			DALYs rate		
	1990 (per 100k)	2019 (per 100k)	EAPC (%)*	1990 (per 100k)	2019 (per 100k)	EAPC (%)*	1990 (per 100k)	2019 (per 100k)	EAPC (%)*
Global	25.04 (18.51, 31.65)	32.21 (23.73, 40.81)	0.98 (0.95, 1.02)	2.96 (2.76, 3.15)	2.86 (2.61, 3.11)	-0.40 (-0.56, -0.24)	230.83 (208.7, 255.62)	236.85 (209.03, 268.91)	-0.05 (-0.16, 0.06)
Male	22.62 (16.48, 28.6)	30.14 (22.32, 38.15)	1.08 (1.05, 1.10)	3.17 (2.91, 3.44)	3.32 (3.05, 3.67)	-0.04 (-0.17, 0.09)	232.6 (209.3, 260.57)	253.82 (225.76, 287.52)	0.19 (0.09, 0.30)
Female	27.53 (20.37, 34.8)	34.33 (25.26, 43.5)	0.90 (0.87, 0.93)	2.74 (2.48, 2.98)	2.38 (2.1, 2.64)	-0.87 (-1.08, -0.65)	229.02 (203.46, 258.05)	219.45 (187.72, 252.02)	-0.32 (-0.45, -0.19)
High SDI	18.12 (12.26, 24.63)	22.97 (15.94, 30.37)	0.73 (0.68, 0.77)	0.76 (0.74, 0.79)	0.8 (0.72, 0.91)	0.47 (0.28, 0.67)	86.63 (72.51, 104.16)	95.66 (77.95, 117.88)	0.52 (0.45, 0.58)
High-middle SDI	25.29 (18.12, 32.57)	31.7 (22.42, 41.57)	0.99 (0.89, 1.08)	2.13 (1.97, 2.3)	1.42 (1.3, 1.54)	-2.15 (-2.44, -1.85)	177.37 (156.58, 200.72)	143.16 (120.07, 168.96)	-1.03 (-1.17, -0.88)
Middle SDI	26.39 (19.43, 33.31)	37.49 (27.3, 47.6)	1.30 (1.28, 1.33)	3.71 (3.45, 4)	3.23 (2.99, 3.5)	-0.85 (-1.03, -0.67)	282.77 (255.22, 313.04)	271.69 (240, 307.31)	-0.33 (-0.44, -0.22)
Low-middle SDI	28.45 (21.43, 35.24)	33.51 (25.23, 42.11)	0.68 (0.61, 0.75)	3.68 (3.31, 4.07)	3.76 (3.35, 4.17)	-0.09 (-0.31, 0.13)	277.11 (247.55, 310.36)	290.52 (257.55, 326.58)	0.08 (-0.10, 0.26)
Low SDI	22.94 (17.38, 28.22)	26.43 (20.04, 32.82)	0.64 (0.56, 0.72)	4.1 (3.6, 4.61)	3.78 (3.28, 4.29)	-0.30 (-0.38, -0.22)	297.86 (264.34, 331.83)	288.27 (250.44, 327.99)	-0.12 (-0.18, -0.06)
East Asia	19.01 (13.44, 24.52)	21.34 (14.33, 28.71)	0.63 (0.51, 0.75)	2.83 (2.43, 3.28)	1.49 (1.27, 1.72)	-3.11 (-3.62, -2.60)	216.86 (186.75, 248.94)	138.82 (114.95, 165.43)	-1.82 (-2.12, -1.52)

(*Continued*)

TABLE 1 Continued

Location name	Incidence rate			Death rate			DALYs rate		
	1990 (per 100k)	2019 (per 100k)	EAPC (%)*	1990 (per 100k)	2019 (per 100k)	EAPC (%)*	1990 (per 100k)	2019 (per 100k)	EAPC (%)*
South Asia	31.93 (24.26, 39.56)	33.01 (24.86, 41.61)	0.29 (0.19, 0.39)	3.42 (2.9, 3.9)	3.73 (3.23, 4.26)	0.13 (-0.18, 0.45)	259.45 (222.69, 296.9)	280.7 (245.22, 319.24)	0.22 (-0.07, 0.50)
Southeast Asia	29.12 (21.77, 36.21)	39.63 (29.77, 49.81)	1.07 (1.05, 1.10)	5.77 (5.23, 6.41)	4.87 (4.32, 5.49)	-0.81 (-0.91, -0.70)	423.57 (382.04, 475.41)	397.09 (346.97, 450.76)	-0.40 (-0.49, -0.32)
Central Asia	50.13 (39.22, 61.49)	68.14 (52.61, 84.48)	1.22 (1.08, 1.35)	4.06 (3.77, 4.48)	4.67 (4.07, 5.35)	-0.56 (-0.99, -0.13)	319.59 (288.26, 358.36)	375.47 (323.81, 430.05)	-0.21 (-0.51, 0.09)
High-income Asia Pacific	21.2 (14.69, 28.37)	22.14 (15.23, 29.73)	0.28 (0.15, 0.41)	0.93 (0.88, 0.98)	0.29 (0.27, 0.31)	-3.99 (-4.21, -3.77)	86.36 (76.52, 97.81)	49.31 (39.34, 61.82)	-1.90 (-2.04, -1.75)
Oceania	34.2 (26.35, 41.87)	40.11 (30.88, 49.23)	0.41 (0.34, 0.48)	4.25 (3.51, 5.02)	4.51 (3.66, 5.68)	-0.02 (-0.26, 0.22)	334.4 (280.81, 396.92)	371.71 (306.63, 453.44)	0.16 (-0.03, 0.34)
Australasia	11.34 (7.02, 16.14)	15.31 (9.74, 21.57)	0.86 (0.76, 0.97)	0.31 (0.29, 0.34)	0.32 (0.28, 0.36)	0.03 (-0.13, 0.19)	45.31 (36.29, 58.55)	50.12 (38.46, 63.84)	0.18 (0.06, 0.30)
Eastern Europe	41.99 (29.69, 54.53)	62.88 (45.32, 83.31)	1.62 (1.37, 1.86)	1.64 (1.59, 1.7)	1.15 (1.02, 1.28)	-2.63 (-3.12, -2.13)	155.6 (134.25, 182.71)	138.5 (109.54, 174.78)	-1.16 (-1.45, -0.87)
Western Europe	11.05 (6.87, 15.75)	12.15 (7.82, 17.2)	0.28 (0.21, 0.36)	0.39 (0.37, 0.4)	0.23 (0.22, 0.25)	-2.01 (-2.15, -1.87)	53.39 (42.74, 67.17)	43.96 (33.74, 57.12)	-0.81 (-0.87, -0.74)
Central Europe	27.94 (19.89, 36.24)	36.4 (26.17, 48.04)	1.06 (0.95, 1.17)	1.61 (1.56, 1.66)	0.81 (0.7, 0.94)	-2.40 (-2.74, -2.07)	133.47 (119.6, 150.85)	93.29 (76.04, 114.32)	-1.16 (-1.41, -0.91)
High-income North America	20.15 (13.28, 28.48)	18.48 (12.16, 25.1)	-0.67 (-0.84, -0.51)	0.77 (0.75, 0.79)	0.93 (0.87, 0.98)	1.06 (0.92, 1.21)	99.62 (80.47, 122.94)	109.57 (90.99, 132.97)	0.51 (0.34, 0.68)
Andean Latin America	18.79 (13.49, 24.35)	31.14 (22.31, 40.32)	1.74 (1.69, 1.79)	3.4 (3.06, 3.77)	3.03 (2.41, 3.76)	-0.66 (-1.05, -0.28)	253.94 (226.93, 283.13)	255.88 (208.87, 307.02)	-0.19 (-0.49, 0.11)
Central Latin America	35.91 (26.56, 45.91)	61.37 (46.39, 76.24)	1.71 (1.59, 1.83)	3.33 (3.25, 3.42)	5.18 (4.56, 5.86)	1.79 (1.69, 1.89)	292.19 (260.09, 332.24)	449.54 (387.8, 519.44)	1.68 (1.61, 1.76)
Caribbean	29.19 (21.9, 36.77)	46.37 (34.53, 58.34)	1.45 (1.26, 1.64)	3.11 (2.75, 3.56)	4.2 (3.36, 5.18)	1.13 (1.06, 1.21)	257.24 (225.04, 298.73)	354.05 (292.18, 421.64)	1.17 (1.13, 1.22)
Tropical Latin America	28.76 (20.79, 36.81)	37.42 (26.69, 48.07)	0.75 (0.65, 0.86)	2.95 (2.84, 3.06)	1.78 (1.69, 1.87)	-1.94 (-2.25, -1.64)	232.87 (211.66, 258.9)	175.32 (150.1, 207.14)	-1.14 (-1.36, -0.92)
Southern Latin America	15.56 (10.4, 20.89)	19.18 (13.11, 25.51)	0.83 (0.79, 0.87)	1.71 (1.59, 1.82)	1.47 (1.31, 1.66)	-0.70 (-0.84, -0.55)	143.33 (127.25, 161.37)	133.23 (114.15, 154.14)	-0.31 (-0.38, -0.23)
Eastern Sub-Saharan Africa	14.11 (10.12, 18.05)	15.33 (10.97, 19.88)	0.43 (0.33, 0.53)	4.19 (3.45, 4.8)	3.25 (2.68, 3.83)	-1.06 (-1.12, -0.99)	302.76 (254.95, 344.28)	252.13 (213.4, 292.44)	-0.77 (-0.83, -0.72)
Southern Sub-Saharan Africa	34.69 (25.96, 43.43)	42.61 (31.68, 53.32)	0.66 (0.41, 0.91)	4.47 (4, 5.11)	4.52 (3.59, 5.44)	-0.31 (-1.13, 0.52)	319.09 (283.23, 361.91)	335.3 (272.77, 401.31)	-0.07 (-0.71, 0.56)
Western Sub-Saharan Africa	27.3 (20.74, 33.56)	32.79 (24.95, 40.46)	0.93 (0.67, 1.18)	4.89 (4.03, 5.97)	4.34 (3.37, 5.34)	-0.36 (-0.41, -0.30)	346.89 (289.3, 414.73)	327.18 (265.01, 393.23)	-0.15 (-0.20, -0.11)
North Africa and Middle East	25.78 (18.52, 33.24)	47.97 (34.81, 60.75)	2.12 (2.09, 2.15)	2.49 (2.22, 2.77)	2.01 (1.64, 2.46)	-0.86 (-0.99, -0.73)	208.08 (184.15, 235.89)	205.76 (168.46, 251.78)	-0.10 (-0.20, 0.00)
Central Sub-Saharan Africa	15.29 (11.03, 19.48)	18.37 (13.04, 23.7)	0.70 (0.64, 0.76)	4.51 (3.61, 5.44)	3.57 (2.69, 4.5)	-0.86 (-0.91, -0.81)	320.88 (263.95, 383.09)	268.45 (211.58, 329.28)	-0.66 (-0.71, -0.62)

*If the EAPC and its lower boundary of the 95% CI were positive, the rate was considered to increase. Otherwise, if the EAPC and its upper boundary of the 95% CI were negative, the rate was considered to decline.

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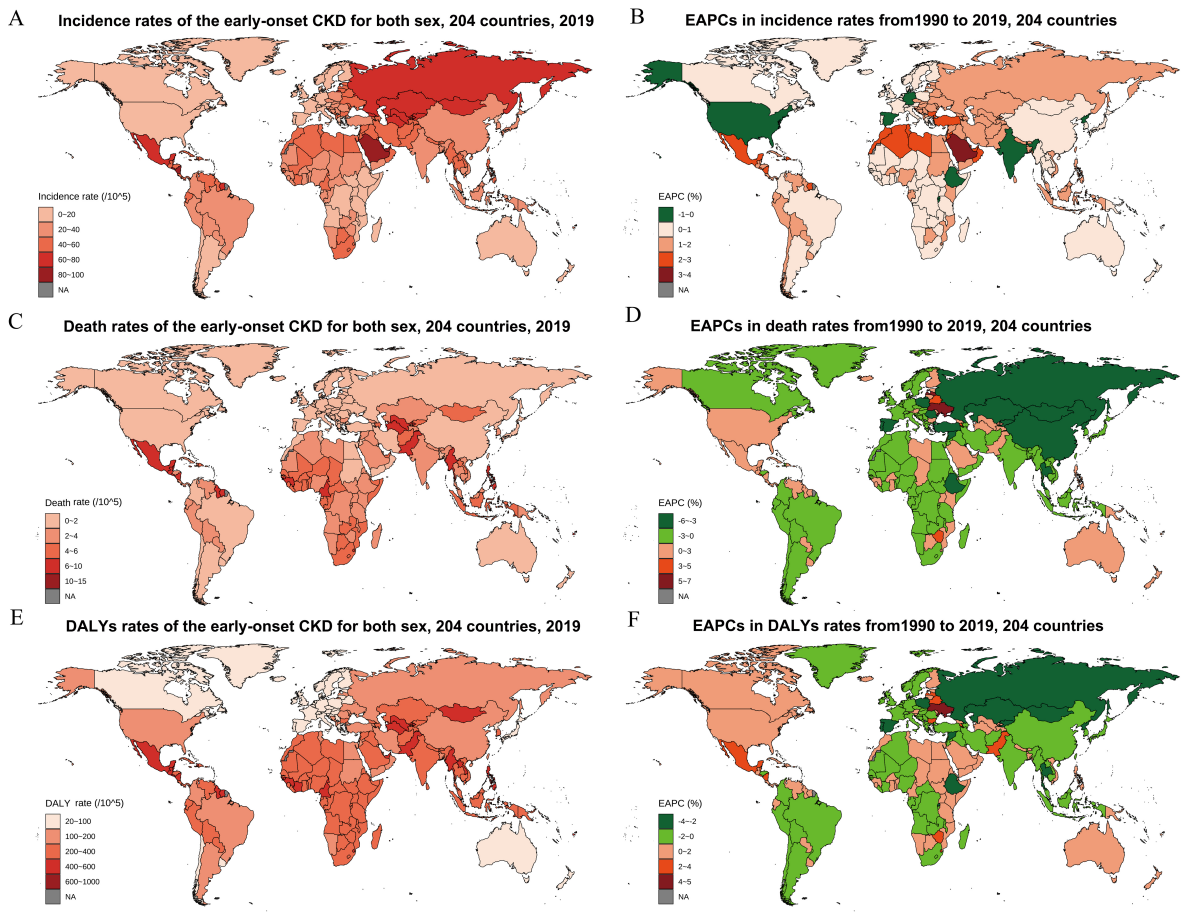

FIGURE 1
The global burden of early-onset CKD for both sexes in 204 countries and territories. **(A)** Incidence rate in 2019; **(B)** The EAPCs in incidence rates from1990 to 2019; **(C)** Death rate in 2019; **(D)** The EAPCs in death rates from1990 to 2019; **(E)** DALY rate in 2019; **(F)** The EAPCs in DALY rates from 1990 to 2019. CKD, chronic kidney disease; EAPC, estimated annual percentage change; DALY, disability-adjusted life year.

rates between men and women were predominantly attributable to the older age groups (35–39 years) (Figure 2). Among all age groups, females had a higher incidence compared with males, and this was reversed in death and DALY with a few exceptions in the early years. Regarding SDI categories, females were estimated to have higher incidence rates than males for all the age groups in all the SDI categories, except for the 15–19 age group in high- and low-SDI countries (Supplementary Figure 2). The sex difference was mainly attributed to the 15–19 years age group for death and DALY rates in low SDI countries (Supplementary Figures 3, 4).

Temporal trend of early-onset CKD from 1990 to 2019

The global numbers of incidence, death, and DALY of CKD in adolescents and young adults have risen during the past 30 years (Supplementary Table 4). Of those, the incidence increased from 5.49 (95% CI 4.06 to 6.94) thousand in 1990, to 9.56 (7.04 to 12.11) thousand in 2019, an increase of 74.13%, markedly higher than the percentage increases for death (30.68%) and DALY (38.83%) numbers.

The incidence rate increased from 1990 to 2019 globally and in all SDI regions (Figure 3A). Detailly, the global incidence rate increased from 25.04 (95% CI 18.51 to 31.65) in 1990, to 32.21 (23.73 to 40.81) in 2019, with an EAPC of 0.98% (0.95% to 1.02%) (Table 1; Figure 3D). Middle SDI Countries exhibited the fastest increase in incidence rate (EAPC 1.30%, 95% CI 1.28% to 1.33%), whereas the EAPC was 0.64% to 0.99% for the other four SDI categories (Table 1; Figures 3A, D). Consistent increasing trends were observed in incidence rates in all age groups globally and by SDI categories and sex (Supplementary Figures 5A, 6A, 7A). All 21 regions exhibited an increase in incidence rate, except High-income North America, which had an EAPC of -0.67% (95% CI -0.84% to -0.51%) (Table 1). North Africa and Middle East had the fastest increase in incidence rate (EAPC 2.12%, 95% CI 2.09 to 2.15) for early-onset CKD between 1990 and 2019, followed by Andean Latin America (1.74%), Central Latin America (1.71%), and Caribbean (1.45%) (Table 1). At the country level, 194 of 204 countries exhibited an estimated increasing temporal trend in incidence rate (Figure 1B; Supplementary Table 1). Saudi Arabia, Bahrain, and Morocco had the fastest increases with an EAPC of 3.06%, 2.81%, and 2.71%, respectively (Figure 1B; Supplementary Table 1).

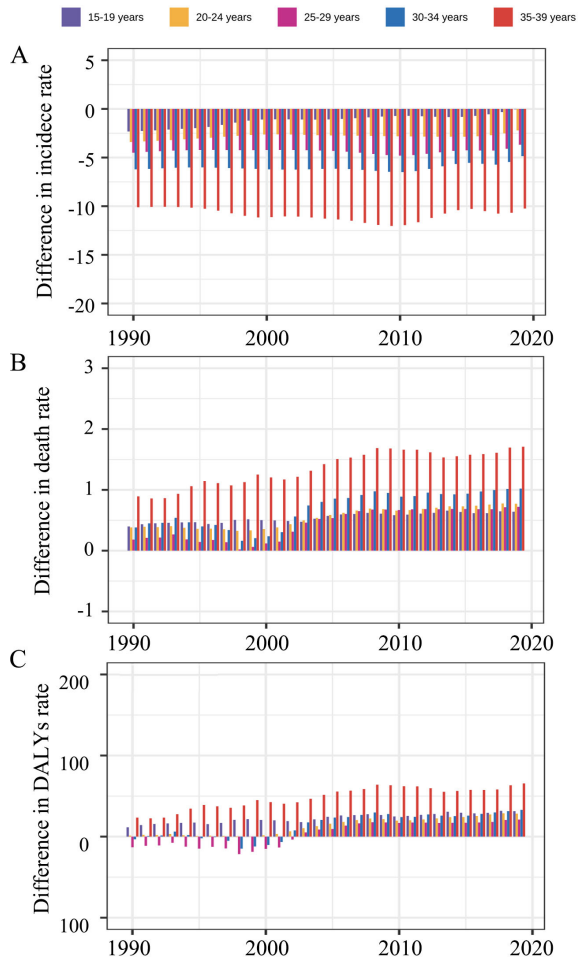

FIGURE 2
The difference in **(A)** incidence rate, **(B)** death rate, and **(C)** DALY rate between men and women by age groups globally, from 1990 to 2019. The difference was calculated using the age-specific rate in males minus that in females, with a difference higher than 0 meaning men have higher rates. DALY, disability-adjusted life year.

Conversely, there was a significant temporal decreasing trend in death rate (EAPC -0.40%, 95% CI -0.56% to -0.24%) and a non-significant decrease in DALY rate (-0.05%, -0.16% to 0.06%) at the global level from 1990 to 2019 (Table 1; Figures 3B–D). The death rate and DALY rate showed the fastest declines in countries with high–middle SDI (Table 1; Figure 3D). There was consistent geographical region variation in the temporal changes of death rate and DALY rate (Table 1). For example, most regions exhibited declines in the death rate and DALY rate over time (e.g., East Asia, High-income Asia Pacific, and Eastern Europe) whereas three regions (Central Latin America, Caribbean, and Australasia) showed significant increasing trends, and four regions (South Asia, Oceania, Australasia, and Southern Sub-Saharan Africa) had non-significant changed trends (Table 1). Among the 204 countries included in the study, 84 and 140 had an estimated increasing temporal trend in death rate and DALY rate, respectively (Figures 1D, F; Supplementary Tables 2, 3). Ukraine had the highest increasing trends of DALY rate (EAPC 4.07%) and death rate (6.85%) from 1990 to 2019. Regarding age groups, the death and DALY rates consistently decreased over time in all age groups, especially in high-middle or middle SDI countries (Supplementary Figures 5B, C, 6B, C, 7B, C). However, significant increasing trends were found in 25–39 age groups within high SDI countries. Furthermore, in low or low-middle SDI countries, females exhibited significant temporal decreasing trends in death and DALY rates for early-onset CKD in all age groups, whereas males had a significant temporal increase in the rates in the 25–39 years group.

Association of early-onset CKD burden with SDI

The non-linear association between the SDI and regional incidence, death, and DALY rates was examined using Gaussian process regression (Supplementary Figure 8). It resembled an asymmetric inverted V-shape association between the SDI and early-onset CKD incidence rates (Supplementary Figure 8A). The death and DALY rates both decreased with SDI levels (Supplementary Figures 8B, C). In terms of the correlation between the EAPCs and SDI (2019) at the national level, an asymmetric inverted V-shape was observed between EAPC for incidence rates and SDI (2019) (Figure 4A). The fitted curve peaked when the SDI approached 0.70, with a significant positive association found ($\rho = 0.449$, $p < 0.001$) when the SDI was limited to below 0.70, and a significant negative correlation observed ($\rho = -0.455$, $p < 0.001$) for SDI above 0.70. No apparent correlation was found in death and DALY in adolescents and young adults (ρ for death -0.04, $p = 0.576$; ρ for DALY 0.029, $p = 0.687$) (Figures 4B, C).

Attributable risk factors for DALY in early-onset CKD

Globally, DALY for CKD can be attributed to seven risk factors including lead exposure, high temperature, diet high in sodium, low temperature, high body-mass index (BMI), high fasting plasma glucose (FPG), and high systolic blood pressure (SBP) (Figure 5; Supplementary Table 5). In 2019, high SBP was the highest attributable risk factor with a proportion of 28.03%, followed by high FPG (18.27%), and high BMI (7.78%). Similar exhibits were observed in different regions divided by SDI, and were mostly consistent in males and females (Figure 5; Supplementary Table 5). By sex, the proportions of most of the global attributable risk factors in males were markedly higher than those in females, except for the high BMI (proportion for female 8.34%, for male 7.30%). The contribution of different risk factors varied with age groups (Supplementary Figure 9). First, high SBP and high FPG were the primary factors for all age groups. Second, high BMI did not contribute to early-onset CKD except in the age group of 35–39 years. Third, for the populations in the 15–19 and 20–24 years groups, only four risk factors (high SBP, high FPG, low temperature, and high temperature) were attributable for the CKD. In addition, the proportion of risk factors contributing to early-onset CKD that was attributable to high SBP and high BMI

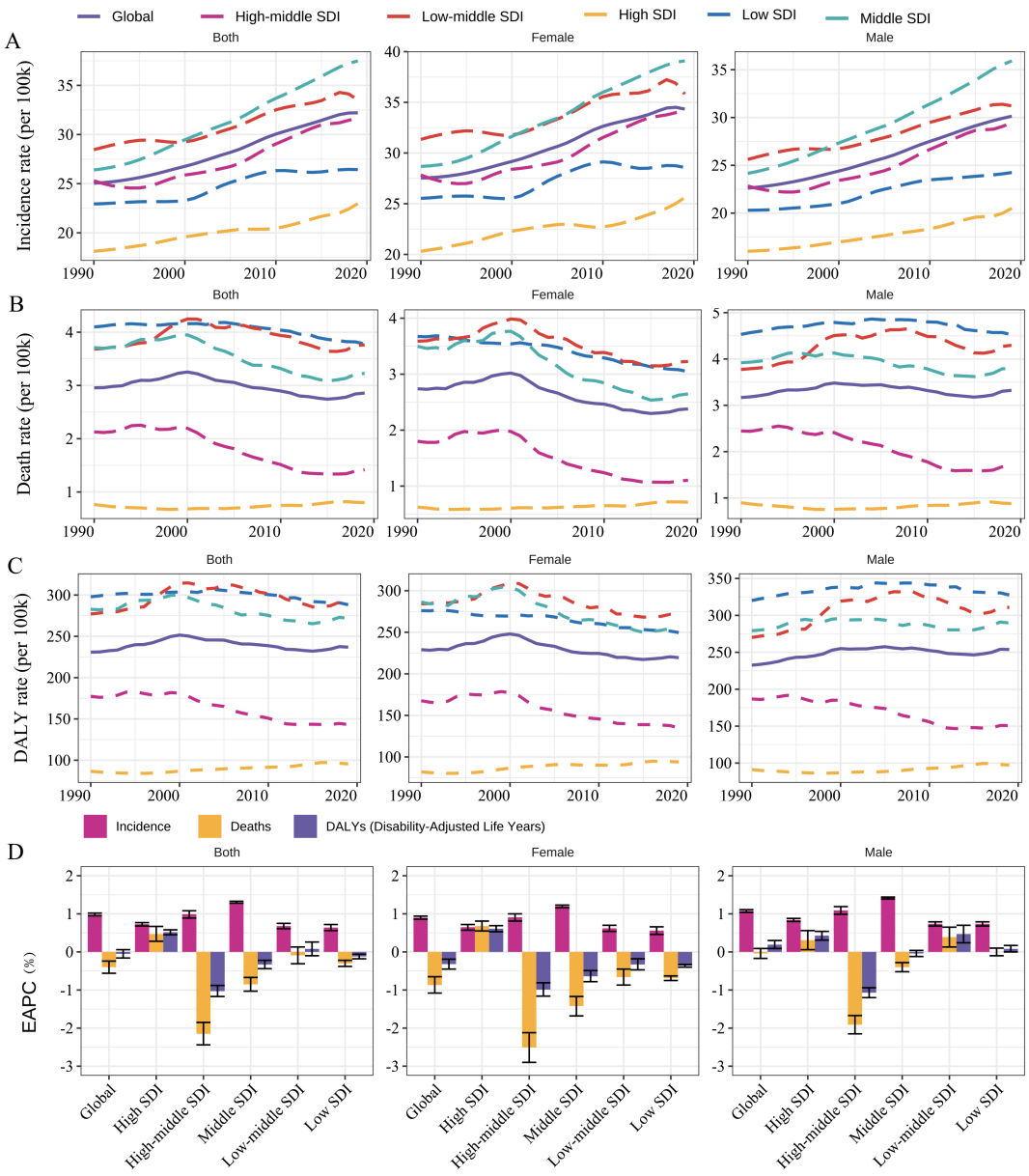

FIGURE 3
Temporal trend of age-specific **(A)** incidence rate, **(B)** death rate, and **(C)** DALY rate for the early-onset CKD globally and by SDI and sex, respectively, from 1990 to 2019. Panel **(D)** show EAPCs of age-specific incidence, death, and DALY rates by SDI and sex, respectively, from 1990 to 2019. DALY, disability-adjusted life year; CKD, chronic kidney disease. EAPC, estimated annual percentage change.

both increased while the proportion of high FPG decreased from 1990 to 2019 (Supplementary Figure 10).

Discussion

This study provides a comprehensive estimation of the burden of CKD among adolescents and young adults. The primary findings are as follows: first, from 1990 to 2019, the global age-specific incidence rate of early-onset CKD showed a significant upward trend, while the death rate exhibited a notable downward trend, and the DALY rate remained stable. Second, in 2019, countries with a middle SDI had the highest incidence rates and the fastest increasing trend in incidence. Low and low-middle SDI countries had the highest death and DALY rates for CKD, while countries with a high-middle SDI experienced the fastest decline in death and DALY rates. Third, the global age-specific incidence rate is higher in women than in men, whereas the death rate and DALY rate are higher in men. Fourth, the burdens of CKD were proportional to age in adolescents and young adults. Fifth, the most contributive risk factors for early-onset CKD were high SBP, high FPG, and high BMI, and patterns of all attributable risk factors varied across regions, age groups, and sexes.

From 1990 to 2019, the global age-specific incidence rate of early-onset CKD exhibited a significant upward trend, while the

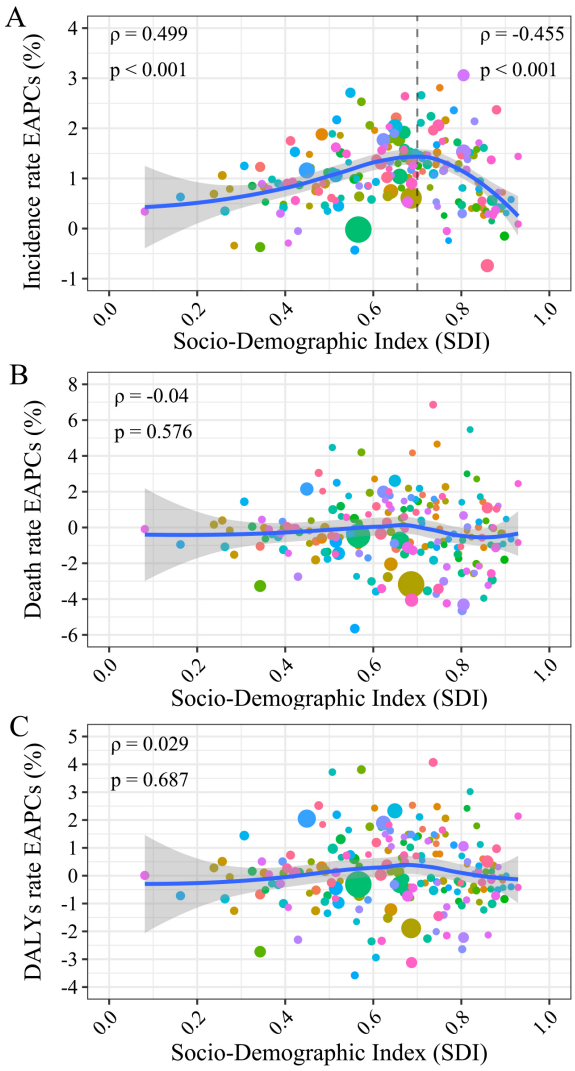

FIGURE 4
The correlation between the EAPCs of incidence/death/DALY and the SDI in 2019. The size of the circle is increased with the number of incidence, death, and DALY cases of early-onset CKD. **(A)** The EAPCs of incidence rate; **(B)** The EAPCs of death rate; **(C)** The EAPCs of DALY rate. EAPC, estimated annual percentage change; DALY, disability-adjusted life year; SDI, socio-demographic index; CKD, chronic kidney disease.

age-specific death rate showed a marked decline. Researches indicate that changes in lifestyle and environmental factors (such as unhealthy diets and rising obesity rates), and advancements in medical diagnostic technologies contribute to the higher incidence rate of early-onset CKD (10, 11), indicating that the relevant institutions should prioritize primary prevention of early-onset CKD and promote CKD health education among the public. Moreover, the age-specific survival rate of early-onset CKD patients has improved, likely attributable to global advancements in medical technology and healthcare, including early diagnosis, effective treatments (such as medication, dialysis, and kidney transplantation), and enhanced patient management and care (12). However, despite the declining death rate, the age-specific DALY rate has remained stable. This phenomenon reflects that early-onset CKD continues to impose a significant long-term health burden, leading to persistent functional loss and decreased quality of life. Therefore, the stable DALY rate indicates a shift in the burden of disease, underscoring the need for ongoing global focus on prevention strategies and long-term management of early-onset CKD.

As indicated by SDI, health outcomes and disease burdens have previously exhibited correlations with socioeconomic development (13). Our findings mirrored this association, showing persistent disparities in early-onset CKD burden across SDI categories. An inverse V-shaped relationship between SDI and age-specific incidence rate, and a descending trend between SDI and age-specific death and DALY rates, align with prior CKD burden distribution patterns (14). The highest age-specific death and DALY rates were found in countries with low and low-middle SDI, primarily attributed to their limited CKD care quality and drug/surgery access (15). An universal health coverage research showed that lower SDI countries have shifted from communicable to non-communicable diseases and injuries, outpacing health system advancements (16). Hence, lower SDI countries should promptly draw upon experiences from developed nations to formulate policies and invest in public welfare for secondary prevention and healthcare for non-communicable diseases, thereby reducing the death and DALY rates of early-onset CKD. In countries with middle SDI, the age-specific incidence rate of early-onset CKD is highest and increasing the fastest, possibly due to increased and exacerbated CKD risk factors attributable to rapid social and economic changes. Research showed that these middle SDI countries have experienced faster socioeconomic development since 2000 (17), but poor lifestyle and medical intervention contribute significantly to the increase in hypertension and diabetes burden, major risk factors for early-onset CKD (18). Additionally, advancements in CKD screening technology and improvements in registry systems may also contribute to the increased incidence of early-onset CKD in these countries (19). Conversely, in countries with high-middle SDI, the death and DALY rates of early-onset CKD are decreasing more rapidly. This may be attributed to more stable social development, rapid improvements in healthcare resources, and increased public awareness of diseases. A study linking access to and quality of healthcare services with overall development supports this view (20). Therefore, middle SDI countries in the period of rapid transition should draw on the experiences of high SDI countries, focusing more on primary prevention (21).

The global burden of early-onset CKD also varied by gender and age. Our study showed that the age-specific incidence of early-onset CKD was higher in women. This sex-based disparity, predominantly observed in individuals aged 35–39, may be attributed to conditions like acute pyelonephritis and pregnancy-related nephropathy in women of child-bearing age, linked to CKD (22, 23). Additionally, the incidence rate among women is more pronounced in low SDI countries, likely due to limited healthcare access and poor metabolic health (24). Research also showed that inadequate lifestyle control during reproductive years

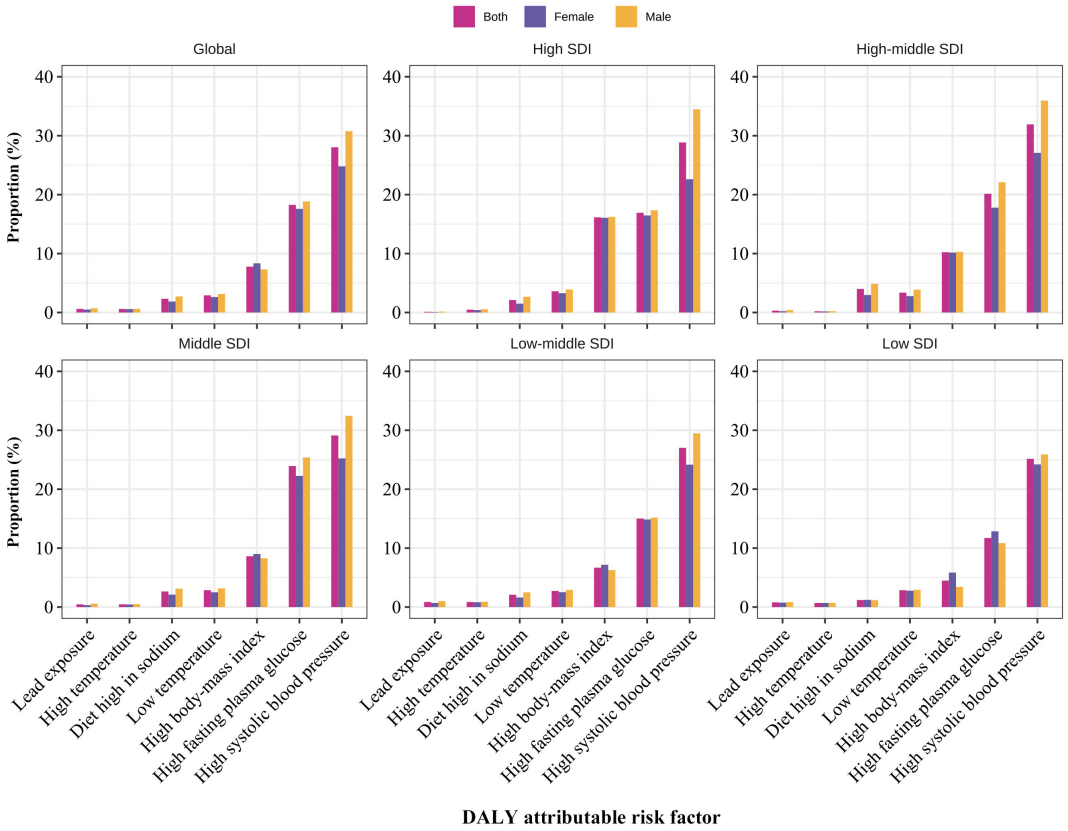

FIGURE 5
The proportion of early-onset CKD DALYs attributable to 7 risk factors classified by sex and SDI regions, respectively in 2019. DALY, disability-adjusted life year; CKD, chronic kidney disease.

may lead to pregnancy-related hypertension, hyperglycemia, and hyperlipidemia, increasing CKD prevalence (25). Thus, effective early-onset CKD prevention in women is crucial. However, men had higher age-specific death and DALY rates. This might result from a higher incidence of congenital kidney and urinary tract anomalies (26, 27). Additionally, research shows that younger individuals may be more susceptible to congenital or genetic factors. In our study, men aged 15-19 exhibited considerably higher incidence, death, and DALY rates than women in low-SDI regions, suggesting that greater attention should be given to the burden of early-onset CKD in younger males in these areas. Relevant policy departments should adjust early-onset CKD prevention and intervention measures based on gender, age, and region, especially among vulnerable populations. For example, targeted programs addressing pregnancy-related CKD risks for women and specialized education and prevention programs for adolescents should be implemented.

Regarding primary prevention, our study showed that high SBP, high FPG, and high BMI were the three major risk factors leading to early-onset CKD. As reported, hypertension is highly prevalent and is the primary risk factor for CKD (28). Also, strong evidence has indicated that high SBP increases the risk of CKD (29, 30). Similar results were found in our analysis that high SBP was the main contributor to the burden of early-onset CKD and increased globally from 1990. In addition, researchers found that if the prevalence of high SBP was reduced relatively by 25% by 2030, the deaths from CKD would be reduced by 0.8 thousand for adults (29). Moreover, the global prevalence of high SBP was generally higher for men than women (29, 30). Our analysis supported this finding, with a high SBP contributing more to the burden of early-onset CKD in men than in women. In addition, the proportional DALY for high SBP had significantly increased from 1990 to 2019, further indicating the importance of preventing hypertension, especially in men.

Meanwhile, in our analysis, high FPG was the second main contributor to the burden of early-onset CKD in 2019, with a higher attributable burden in men, consistent with the KDIGO clinical practice guideline (31). Moreover, studies have shown that with the occurrence of obesity in younger ages, the prevalence of diabetes is likely to continue to increase substantially (32, 33). This is supported by global data showing a significant increase in the incidence of type 2 diabetes among adolescents and young adults (15–39 years of age) worldwide between 1990 and 2019 (34). A study from China also showed that all-age prevalence of diabetes rose from 3.7% to 6.6%, and death rates for all-age diabetes and diabetes-related CKD increased by 63.5% and 33.3%, respectively, from 1990 to 2016 (35). In addition, our analysis showed that high BMI was the third main contributor to the burden of early-onset CKD, with a higher attributable burden in women. This result aligned with the opinion of a review that obesity is at the epicenter

of the global CKD problem (36). Between 1990 and 2017, the prevalence of overweight and obesity has increased globally in all age groups and regions (37). Moreover, the global deaths and DALYs attributable to high BMI were estimated to have more than doubled (38). Therefore, men should focus on stricter blood pressure and blood sugar control, while women should prioritize weight loss, considering different risk factors.

Similarly, across all age groups included in our study, high SBP and high FPG were the leading causes of early-onset CKD DALYs in 2019, with high BMI also contributing significantly to DALYs in the 35-39 age group. These findings further emphasize the importance of managing these three leading risk factors and developing gender- and age-specific strategies for CKD primary prevention. Across all SDI regions, the main attributable risk factors for early-onset CKD maintain consistent contribution ranks, highlighting the necessity of cohesive global policies. High SBP, high FPG, and elevated BMI remain significant factors in all SDI categories. The experience of low- and middle-income countries indicates that a large number of asymptomatic and undiagnosed individuals contribute to the burden of various non-communicable diseases, including diabetes, hypertension, and advanced CKD (39). Nonetheless, studies have demonstrated that early asymptomatic CKD can be identified through simple tests. Identifying and treating high-risk individuals with CKD through case finding can provide benefits at a reasonable cost (11). Therefore, it is necessary to reduce the underreporting of early-onset CKD by expanding screening coverage, improving data collection and reporting systems, standardizing diagnostic criteria, strengthening monitoring, and increasing risk factor screening programs (40). Training healthcare professionals and conducting public health education campaigns may also enhance awareness and improve early detection. Additionally, strengthening global health management and emphasizing proactive health concepts are crucial for addressing these consistent chronic disease risk factors.

Study limitations

Our study is subject to several limitations. First, estimates of the burden of early-onset CKD largely depend on the availability and quality of data from the GBD 2019. Some countries, especially low-income countries, may lack original/raw data, which could hinder GBD researchers' ability to make accurate estimates. Second, differences in diagnostic and detection protocols for early-onset CKD across countries and over time may affect the comparability of the results. Given the uncertainty of the original data, caution should be exercised when interpreting the trends in the burden of early-onset CKD found in this study. Third, a narrow focus on significance testing may overlook the clinical relevance of these findings. To mitigate this limitation, we advocate for the development and implementation of multiple analytical methods to broaden and validate the results of this study.

Conclusions

Although the age-specific death rate of early-onset CKD significantly decreased from 1990 to 2019, the age-specific incidence rate continued to rise and the age-specific DALY rate remained stable. The burdens of early-onset CKD varied by gender, age, and region. Additionally, high SBP, high FPG, and high BMI were the primary risk factors contributing to the burden of early-onset CKD. Targeted primary and secondary prevention measures, as well as healthcare interventions, should be developed based on these risk factors, considering different ages, genders, and regions.

Data availability statement

Publicly available datasets were analyzed in this study. This data can be found here: Global Health Data Exchange GBD results tool (http://ghdx.healthdata.org/gbd-results-tool).

Author contributions

PS: Conceptualization, Funding acquisition, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing. XM: Data curation, Resources, Software, Writing – original draft, Writing – review & editing. TS: Validation, Writing – review & editing. YC: Formal analysis, Validation, Visualization, Writing – original draft, Writing – review & editing. XY: Methodology, Software, Validation, Writing – original draft, Writing – review & editing. ZS: Validation, Writing – original draft, Writing – review & editing. XZ: Writing – original draft, Writing – review & editing. LT: Writing – original draft, Writing – review & editing. ZM: Resources, Visualization, Writing – original draft, Writing – review & editing. ZW: Conceptualization, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2024.1389342/full#supplementary-material

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Childhood onset C3 glomerulopathy: recurrence after kidney transplantation—a case series

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Background: C3 Glomerulopathy (C3G) is a complement-mediated disease, with predominant C3 deposits, where pathogenic genetic variants in complement system components and circulating autoantibodies result in loss of control of the alternative pathway, have been described. A high incidence of disease recurrence including graft failure has been reported after kidney transplantation (KTx). Currently treatment modalities for preventing and treating post KTx C3G recurrence (plasma exchange, rituximab and eculizumab) in adults have yielded inconsistent results. Data on post KTx C3G recurrence in childhood-onset C3G is still unknown.
Methods: A comprehensive case study of patients diagnosed with C3G as children or adolescents, who underwent KTx between the years 2015–2023. Data collected included complement workup, treatment modalities, and outcomes.
Results: 19 patients with C3G were identified during the study period. Five patients developed ESRD and received a kidney transplant. C3G recurrence was diagnosed post KTx in 100% of patients. Graft function improved in 3 of these patients (two with anti-factor H antibodies) after eculizumab treatment, one patient reached graft failure 9 months after transplantation despite eculizumab, recieved a second successful transplantation with pre-emptive eculizumab treatment and one patient showed histologic signs of disease recurrence without clinical signs.
Conclusions: C3G recurrence after KTx in patients diagnosed as children or adolescents may be higher than previously described. Treatment with eculizumab is beneficial in some patients. New treatments are needed for improving post-transplant outcome in patients with C3G.

KEYWORDS

C3 glomerulopathy, kidney transplantation, disease recurrence, complement, case series

Introduction

C3 glomerulopathy (C3G) is a complement-mediated disease, with predominant C3 deposits. C3G was re-classified in 2013 and is subdivided to: dense deposit disease (DDD) and C3 glomerulonephritis (C3GN), reflecting kidney biopsy electron microscopy findings (1). According to some published data DDD tends to be a more aggressive disease than C3GN, leading to a higher rate of end stage kidney disease (ESKD) (2, 3). Other reports showed that clinical factors such as kidney function and severity of proteinuria were related to worse long term outcomes (4).

In C3G the pathogenesis is linked to mutations and risk haplotypes in several complement system components and circulating autoantibodies, resulting in the loss of control of the complement system alternative pathway (5). Autoantibodies stabilizing C3 and C5 convertases, including C3 nephritic factor (C3NeF), C5 nephritic factor (C5NeF), factor H mutations or anti-factor H autoantibodies result in a stable convertase, resistant to decay, leading to persistent complement activation. These, together with other mutations and antibodies were described in patients with C3G.

Dysregulation of the complement alternative pathway causes ongoing complement activation, with increased C3 turnover, C3 consumption and systemic low C3 levels. Deposition of complement proteins along the capillary walls of the glomerulus result in mesangial and endocapillary proliferation and capillary-wall remodeling (5, 6).

In adults a high incidence (between 30% and 77%) of C3G disease recurrence has been reported after kidney transplantation (KTx), leading to graft failure in 17%–50% of those affected (7). In a series of 21 transplanted patients with C3GN, diagnosed at a median age of 20.8 years (8), 14 patients (67%) developed recurrence of C3GN in the transplanted kidney at a median time of 28 months from transplantation, and graft failure in 50% of them, at a median time of 77 months from transplantation. Circulating C3 levels prior to transplantation were available for only some of the patients in this cohort, but they were normal in the five patients without recurrence. Other cohorts also reported a high disease recurrence rate, with graft loss in more than 50% of those with disease recurrence (9, 10), which appeared to occur more often in DDD (11).

Therapeutic alternatives of C3G in native kidneys include ACE inhibitors or angiotensin receptor blockers alone in patients with mild disease, to immunosuppression regimens including corticosteroids and mycophenolate mofetil in patients with nephrotic range proteinuria or impaired kidney function (12). In patients with more severe disease or in non-responders to this drug combination, complement inhibitors may be beneficial (13, 14).

Current treatment modalities for C3G recurrence after KTx include removing autoantibodies and repleting factor H using plasma exchange, preventing autoantibodies formation using rituximab, and trying to block terminal complement activation with eculizumab, or other experimental complement blocker agents. The literature is inconsistent regarding the results of these interventions (7, 11, 15, 16).

No guidelines exist regarding the need for prophylactic treatment or conditioning before and after KTx in individuals with C3G. Potential predictive factors for disease recurrence include certain genotypes, the presence of autoantibodies, and the current status of complement dysregulation. Still, no specific data are available to guide decisions prior to transplantation, and current recommendations are based on expert opinion and case reports (8, 17).

Regarding childhood onset C3G and disease recurrence after kidney transplantation—one case report describes pediatric transplant related C3G: a patient with a diagnosis of DDD in the native kidneys and recurrence after transplantation, who was treated with rituximab and eculizumab (15), In this patient the treatment effectively inhibited the terminal complement cascade but only partially prevented disease progression. Other cases of pediatric onset C3G were described along with adult cohorts. The disease recurrence rate after KTx in childhood onset C3G, the risk factors for recurrence, and the optimal treatment of recurrence are still unknown. Therefore, this study aimed to describe a case series of transplanted patients with childhood onset C3G, including treatment modalities and outcomes.

Methods

For this case series study, data were collected of patients diagnosed with C3G as children or adolescents in 2 large pediatric nephrology units and underwent a KTx between the years 2015–2023. All patients underwent a native kidney biopsy at presentation. A for cause kidney biopsy in the native or transplanted kidney was performed for indication (proteinuria, increase of serum creatinine). All kidney biopsies were interpreted by a nephropathologist. The biopsy interpretation was based on the current definition of C3G including light microscopy findings of glomerulonephritis with predominant C3 deposits and findings of DDD or C3GN in electron microscopy (1).

For all patients, a detailed medical history, as well as clinical, laboratory and histopathological data, were available regarding native kidney and transplanted kidney disease. All patients underwent a functional and genetic complement system workup at the molecular otolaryngology and renal research laboratories in the University of Iowa—including—levels of factor H, factor I, factor D, anti-factor H antibodies, anti-Factor B antibodies, C3Nef, C5Nef, terminal complement activity markers, and other complement biomarkers. Genetic tests for CFH, CFI, MCP, CFB, CFHR5, C3, THBD, DGKE, PLG, ADAMTS13, MMACHC, G6PD, WT1including copy number variation screening of the CFH-CFHR5 region using multiple ligation dependent probe amplification.

Post KTx C3G recurrence was determined according to histology findings of transplanted kidney biopsy showing features of C3G as defined by Pickering et al. C3 Glomerulopathy: consensus report (1). The study was approved by the local Helsinki committee and the Israel Ministry of Health.

Results

During the study period, out of 19 patients with C3G who were actively followed, five reached ESKD and underwent KTx. Patients' characteristics are described in Table 1. Initial C3G diagnosis median age was 16 years (range: 7.5–17). According to biopsy at presentation, three patients had features consistent with DDD. Electron microscopy was not available for the other two patients.

Four patients were initially treated with immunosuppression for their native kidney disease, including corticosteroids, cyclophosphamide and mycophenolate mofetil in 3 patients and plasmapheresis in one patient. In spite of that, all reached ESKD. Two patients presented with dialysis dependent kidney failure, one did not respond to immunosuppressive treatment and in the other one treatment was not initiated due to diffuse global sclerosis in the native kidney biopsy. The mean time from the initial diagnosis to ESKD was 4.6 (range: 0–17) years.

All patients had low C3 levels at initial presentation. Two patients were positive for anti factor H antibodies (titers of 3,087 AU and 1960AU, normal range <200 AU), one of them was also positive for both C3Nef and C5Nef. Another patient was positive for both C3Nef and C5Nef, one patient had low Factor B levels (Table 2). Genetic tests were positive for a pathogenic variant only in one patient with factor H antibodies who had a homozygous deletion of the CFHR3-CFHR1 gene. Two other patients had findings described as variants of unknown significance, and one of them had also CFH gene associated risk alleles (Table 2).

All patients underwent KTx, one of them recieved 2 deceased donors KTx's during the study period. All other KTx's were from living non-related donors. The median age at first KTx was 17 (range 9–30) years. All patients remained with low C3 levels at the time of transplantation (as shown in Table 2). Disease course after KT reflected by creatinine and proteinuria values is shown in Figure 1.

C3G transplanted patients were treated according to local protocol including induction therapy using Basiliximab (for the first transplantation) and anti thymocyt globulin (ATG) for second transplantation, and triple immunosuppression regimen including tacrolimus, mycophenolate mofetil and corticosteroids. Maintenance immunosuppression included tacrolimus (trough level 5–7 ng/ml), mycophenolate mofetil in all patients and low dose corticosteroids. One patient who was known to have anti factor H antibodies (patient 3) was treated with plasmapheresis and Rituximab prior to and after KTx, before recurrence was diagnosed. Patient 4 who showed signs of complement dysregulation on immunologic workup– received Eculizumab post transplantation. All patients had a recurrence of C3G in the transplanted kidney. Pathological findings in all the instances of recurrence were consistent with DDD. Immunofluorescence staining was positive for C3 deposits in all the patients. Patient 2 had only histologic signs of C3G recurrence but no clinical signs. His biopsies were performed two and five months after KTx due to mild T-cell mediated rejection and polyoma BK nephropathy, respectively. Both biopsies showed C3 deposits by immunofluorescence and electron dense deposits in electron microscopy.

Four patients had clinical signs of disease recurrence including microscopic hematuria, proteinuria, and elevation of serum creatinine. Patient 1, the youngest in the cohort, had severe and rapid disease recurrence which did not respond to eculizumab treatment, and reached graft failure within 9 months after first transplantation. Almost 3 years after her first KTx, she had a second KTx with prophylactic eculizumab treatment, with no signs of recurrence 12 months after. This patient was positive for C3Nef and C5Nef showing very high titers before 1st and 2nd transplantation (77%, 73% and 79%, 91% respectively (normal range <20%).

Patient 3, who was positive for anti-factor H antibodies, was treated before and after transplantation with weekly plasmapheresis and rituximab. Recurrence was observed 18

TABLE 1 Characteristics of the patients.

	Patient 1		Pateint 2	Pateint 3	Pateint 4	Pateint 5
Gender	F		M	F	M	M
Age at presentation (years)	7.5		15	12	17	16
Native kidney biopsy	C3G		DDD	DDD	DDD	C3G
EM available	No		Yes	Yes	Yes	No
Treatment for native kidney disease	CS, CYC		CS, CYC, MMF	CS, CYC, MMF, PP, RTX	CS, CYC, MMF, CsA	None
Time to ESKD (years)	0		2	8	13	0
KT #	1	2	1	1	1	1
Age at KT (years)	9	12	17	20	30	16.5
Time from KT to recurrence (months)	2	No recurrence	2	18	8	2
Clinical/laboratory signs of disease recurrence	H, P, elevated SCr	none	none	H, P, elevated SCr	P, Hi SCr	P, elevated SCr
Transplanted kidney biopsy	DDD	Not preformed	DDD	DDD	DDD	C3G (EM unavailable)
Treatment of recurrence	ECU	Prophylactic ECU	none	PP, RTX, ECU	RTX, ECU	CS, ECU
Post KT follow up (months)	45	12	45	43	26	11
SCr at last follow up (mg/dl)	On dialysis	0.56	1.3	1.26	1.19	1.9
UPCR at last follow up (mg/mg)	anuria	0.2	0.13	0.26	0.9	0.16
Highest UPCR (mg/mg)	11.4	0.2	0.25	9.6	4.8	0.57

DDD, dense deposit disease; CS, Corticosteroids; CYC, Cyclophosphamide; MMF, Mycophenolate Mofetil; PP, Plasmapheresis; CsA, Cyclosporin A; ESKD, end stage kidney disease; KT, Kidney transplantation; H,hematuria; P, proteinuria; SCr, serum creatinine; UPCR, urine protein/creatinine ratio.

TABLE 2 Complement system biomarkers and genetic analysis.

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
C3 at presentation (mg/dl)[a]	20	62.8	6.6	8.8	19
C3 at KT (mg/dl)[a]	1st KT: 9.3	75.7	10.6	7.6	12.6
	2nd KT: 13.8				
C3 at recurrence (mg/dl)	30	92	7.4	NA	63
C3 at last follow up (mg/dl)[a]	1st KT 21.7	91	9.2	6.6	76
	2nd KT 25				
Complement workup	C3Nef	Low factor B level	Anti factor H antibodies	Anti factor H antibodies	Borderline C3Nef
	C5Nef			C3Nef	
				C5Nef	
Genetic tests	Multiple VUS[b] Risk alleles—3 copies of *CFH*-associated risk alleles	Negative	Homozygous deletion CFHR3-CFHR1	Negative	Negative

NA, not available; C3Nef, C3 nephritic factor; C5Nef, C5 nephritic factor; VUS, variant of unknown significance.
[a]C3- Normal range, 90–180 mg/dl.
[b]C8B, CFH, CFHR5, FCN1, PLG.

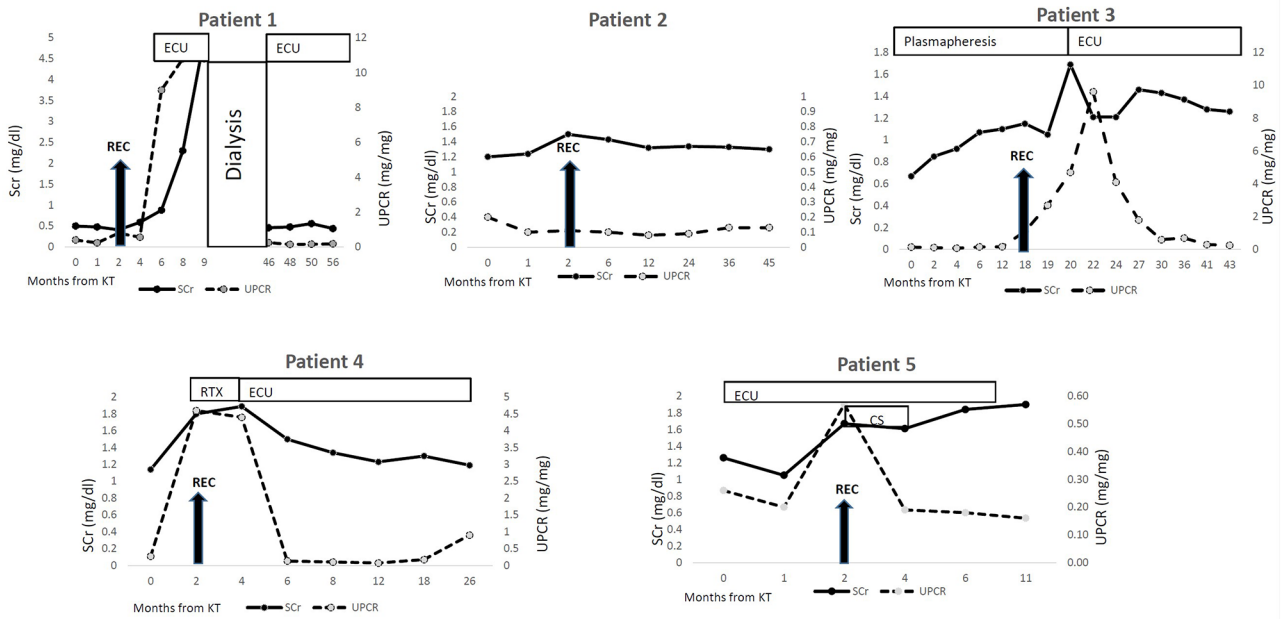

FIGURE 1
Disease course after kidney transplantation. KT- Kidney transplantation REC- recurrence CS- corticosteroids RTX- rituximab ECU-eculizumab SCr-serum creatinine UPCR- urine protein/creatinine ratio.

months after transplantation and treatment was switched to eculizumab. Patient 4, who was also positive for factor H antibodies, had signs of disease recurrence 8 months after transplantation. He was initially treated with rituximab which did not impact the proteinuria and treatment with eculizumab was initiated. Eculizumab treatment for both these patients was beneficial: proteinuria improved and kidney function normalized. Patient 3 reached full remission with no proteinuria or hematuria, patient 4 had normal serum creatinine and mild proteinuria at last follow up (16 months after treatment initiation).

Patient 5 was treated with Eculizumab since transplantation due to evidence of ongoing complement dysregulation. Two months after transplantation, there was evidence of disease recurrence. He received corticosteroid pulse therapy and continued eculizumab treatment, with normalization of proteinuria and improvement in serum creatinine level. Five months after KTx a second biopsy was preformed due to creatinine elevation—showing chronic transplant glomerulopathy, with no evidence of C3G recurrence.

Discussion

Recurrence of complement mediated kidney disease after KTx is a major concern and a main cause for graft loss. Complement dysregulation can be due to congenital or acquired causes. Like other complement mediated kidney diseases, C3G has a high rate of recurrence after KTx, our experience showed a 100% disease recurrence rate. For other diseases, such as atypical hemolytic uremic syndrome, prophylactic treatment with complement C5 inhibitor (eculizumab) prevents disease recurrence in most patients and dramatically changed outcomes of KTx (18). In contrast, no treatment has been shown to consistently prevent recurrence of C3G. In cases of C3G the mechanism of injury is different from atypical HUS as depositions of C3 split products in the mesangium initiates the inflammatory process. Case reports describe successful treatment with eculizumab in some patients (15, 19, 20), while others report eculizumab failure in preventing C3G recurrence (21). Failure of eculizumab treatment (which prevents C5 activation) may be associated with the presence of C3 nephritic factor, which leads to a predominant C3 (proximal to C5) activation. Analyzing C3Nef-mediated C3 and C5 activation separately could help in selecting the appropriate patients for eculizumab therapy. In our small cohort eculizumab was effective in three out of 5 cases of C3G recurrence, two of them with anti factor H antibodies. Factor H is a key inhibitor of complement overactivation, and mutations or antibodies in this protein lead to atypical HUS or C3GN.

Other treatments such as plasma exchange and rituximab have shown various results and success rates, and may be related to the primary reason for complement dysregulation in each patient (7). For patients who are positive for anti-factor H autoantibodies, these antibody depleting treatments may be reasonable, but from our experience, the patients who were positive for anti factor H antibodies did not reach remission under this treatment, and improved only after changing treatment to eculizumab.

Among the adult population, recurrence rates are estimated as 30%–70%. In a summary of small series and case reports which included a total of 122 patients with post-transplant C3G (73 C3GN and 49 DDD) (7), the authors reported higher allograft loss with plasmapheresis and rituximab compared to eculizumab. Subgroup analysis showed a higher rate of graft loss despite treatment among patients with DDD compared with C3GN.

Recurrence rates among patients who presented with C3G as children or adolescents are still unknown. Our cohort exhibited a recurrence rate of 100%. A suggested explanation might be that the disease penetrance is higher in children leading to an early presentation in childhood and a more aggressive form of disease leading to higher rates of recurrence following transplant. Additional research is needed to understand these differences. Recurrence was symptomatic in four of the five patients with recurrence. One of them, the youngest of the cohort, showed rapid deterioration to ESKD and dialysis. While undergoing dialysis treatments, C3 levels remained very low and repeat complement workup still showed ongoing complement dysregulation, and presence of C3 and C5 nephritic factors. This patient had a second KTx using prophylactic eculizumab treatment with no signs of recurrence during 12 months of follow up. The other two patients who were positive for factor H antibodies also had disease recurrence, which was controlled under eculizumab treatment, similar to previous reported patients (22). Previous studies described few cases of second transplantation after C3G recurrence with various outcomes (11), to our opinion in such cases prophylactic complement blocker treatment should be considered.

The question of to how to address low C3 levels before transplantation remains unanswered. Low C3 levels may indicate ongoing complement activation, but C3 levels do not properly correlate with disease activity: 25% of pediatric C3G patients and about 50% of adult C3G patients present with normal blood C3 levels despite an active disease (23). Measurement of circulating terminal attack complex (C5b-9) levels prior to transplantation, may aid the decision regarding prophylactic treatment as patient with elevated circulating C5b-9 may benefit from eculizumab treatment. In our cohort four patients were treated successfully with eculizumab two had high C5b-9 levels one had normal C5b-9 level and for one C5b-9 level before treatment initiation was unavailable.

In patients with C3G, heterogeneity is observed in the exact underlying complement disorders that cause complement dysregulation and ongoing activation. Understanding the particular complement disorder may aid in risk stratification for disease recurrence. The novel complement system blockers that are currently examined in clinical trials may offer a new horizon for individuals with C3G who undergo KTx (16, 24, 25). A better understanding of the underlying complement disorders will contribute to decision making, specifically, the optimal prophylactic treatment to prevent disease recurrence, if any, for each patient.

Our study has several limitations. First—this is a single center study dealing with a rare complement mediated disease—therefore the number of patients is very small. The second limitation is that complement biomarkers testing is only available only and in a few laboratories around the world—so real time and repeat tests cannot be performed. Third—in our center we do not conduct protocol biopsies—therefore we cannot know at this point whether patient 1 is experiencing subclinical recurrence in the second graft.

Despite the small cohort there are lessons learned from this study—C3G is a heterogeneous disease, and time from presentation to ESKD is variable. Disease recurrence can be asymptomatic and may not need any additional treatment. In cases of symptomatic recurrence or in cases of second transplantation eculizumab or other experimental complement blocking agent may serve as a potential treatment.

Conclusion

C3G recurrence rate after KTx in patients diagnosed as children or adolescents may be higher than described in the adult onset C3G. Treatment with currently available tools is successful in only some patients. New treatments are needed for safe transplantation in the context of C3G and for avoidance of disease recurrence.

Summary at glace

Patients diagnosed with C3 Glomerulopathy as children or adolescents were followed after kidney transplantation. All patients developed disease recurrence after transplantation confirmed by kidney biopsy. Some responded to therapy with improved proteinuria and renal function.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The study was approved by the local Ethics Committee in accordance with GCP guidelines. Due to the retrospective nature, patient/guardian consent was not required.

Author contributions

YB: Conceptualization, Data curation, Investigation, Methodology, Writing – original draft. DL: Writing – review & editing. AD: Writing – review & editing. HA: Writing – review & editing. OH: Writing – review & editing. SL: Writing – review & editing. GH: Writing – review & editing. DL: Writing – review & editing. ST-B: Writing – review & editing. JW-G: Writing – review & editing. AT: Writing – review & editing. RR: Writing – review & editing.

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Conflict of interest

YB declares that she received honoraria for lecturing and participating in advisory board from Novartis and a participant in C3G clinical trials by Novartis and Apellis. RR declares that she received honoraria for lecturing from Novartis.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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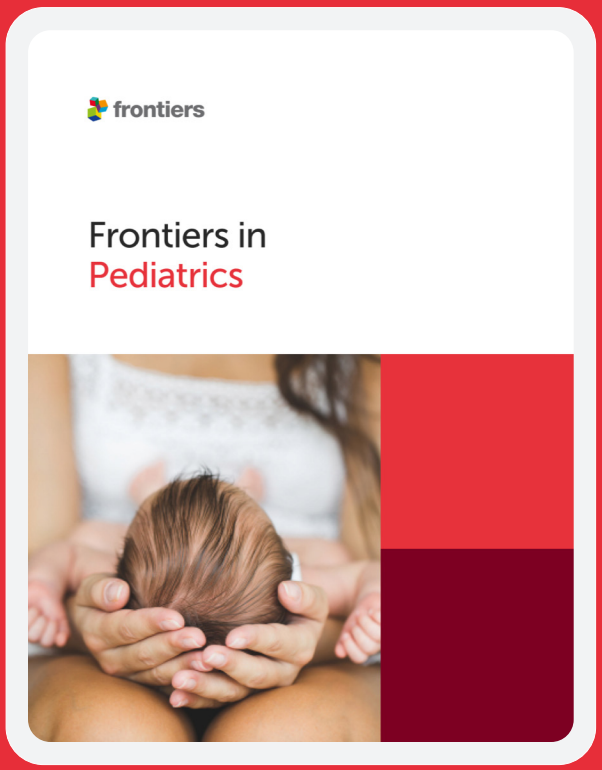

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