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Diagnostic Utility of Galactose-Deficient Immunoglobulin A1 Immunostaining in the Differentiation of Lupus Nephritis and Immunoglobulin A Nephropathy

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Keywords
Lupus nephritis · Immunoglobulin A nephropathy · Galactose-deficient immunoglobulin A1 · KM-55 · Renal biopsy

Abstract
Background: Renal biopsy plays an important role in the establishment of the diagnosis and the management of patients with lupus nephritis. Immunoglobulin A (IgA) nephropathy rarely has been reported in kidney biopsy of lupus patients. Lupus nephritis and IgA nephropathy can be readily diagnosed on renal biopsy when the classic patterns are present. However, atypical patterns can become a diagnostic challenge. Galactose-deficient IgA1 (Gd-IgA1) is a key element in the pathogenesis of primary IgA nephropathy. Glomerular Gd-IgA1 deposits, detected by immunofluorescent staining of KM-55 (a Gd-IgA1-specific monoclonal antibody), are consistently identified in the mesangium of IgA nephropathy but are significantly less or absent in lupus nephritis accompanied by significant IgA deposition. ***Case Presentation:*** Here we report the case of an 11-year-old girl who was recently diagnosed with systemic lupus erythematosus (SLE) and was found to have hematuria and proteinuria. Renal biopsy showed focal mesangial hypercellularity with IgA dominant, "full house" like pattern of mesangial deposition. The biopsy findings present a diagnostic dilemma with the differential diagnosis of IgA nephropathy versus lupus nephritis with atypical immunofluorescence, and IgA nephropathy is favored, in the absence of strong straining of C1q or C3, extraglomerular deposits, tissue antinuclear antibodies, and endothelial tubuloreticular inclusions. However, no detectable glomerular KM-55 staining was seen in the kidney biopsy. ***Conclusions:*** We demonstrate the unique diagnostic utility of immunostaining for KM-55 in a challenging kidney biopsy of an SLE patient with features suggestive of IgA nephropathy. The absence of KM-55 staining excludes IgA nephropathy, supporting a diagnosis of lupus nephritis with atypical immunofluorescence in this patient with SLE.

Background

Renal involvement occurs frequently in systemic lupus erythematosus (SLE). Renal biopsy plays an important role in the establishment of the diagnosis and the management of patients with lupus nephritis [1]. The widely ac-

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cepted classification is the International Society of Nephrology/Renal Pathology Society (ISN/RPS) classification of lupus nephritis [2, 3]. It provides information on the class, severity, activity, and the chronicity of the lupus nephritis. Characteristic pathologic features of lupus nephritis include (1) so-called "full-house" staining, meaning 3 immunoglobulin (Ig) classes (IgG, IgA, and IgM) and both complement components (C1q and C3); (2) strong staining for C1q; (3) tissue antinuclear antibodies (ANA) on immunofluorescence; (4) extraglomerular immune complex deposition by immunofluorescence or electron microscopy; and (5) endothelial tubuloreticular inclusions by electron microscopy [4]. Each feature has a sensitivity ranging from 0.68 to 0.80 and a specificity from 0.8 to 0.96 [4]. Combinations of these pathologic features can assist in distinguishing lupus nephritis from nonlupus nephritis with high specificity and varying sensitivity.

IgA nephropathy has been reported in patients with SLE, although rare [5–12]. IgA nephropathy, the most common primary glomerulonephritis globally, is characterized by glomerular deposition of polymeric IgA1 with aberrant glycosylation at the hinge region O glycans (Gd-IgA1) and the autoantibody formation against this region. Recently, KM-55, a galactose-deficient IgA1 (Gd-IgA1)-specific monoclonal antibody generated by immunizing rats with the human Gd-IgA1 hinge region peptide, has been shown to recognize Gd-IgA1 in renal biopsy tissue on formalin-fixed paraffin-embedded sections from all patients with primary IgA nephropathy and IgA vasculitis but not in other examined disease, such as lupus nephritis, cirrhosis, or hepatitis C-related glomerular disease [13]. However, the specificity of KM-55 staining for primary IgA nephropathy and IgA vasculitis was questioned in more recent studies demonstrating KM-55 staining in incidental IgA deposition, secondary IgA nephropathy, Staphylococcal infection-associated glomerulonephritis, and lupus nephritis albeit with weaker staining than in primary IgA nephropathy [14–16]. Importantly, what is consistently observed in these studies is negative [13, 15] or significantly lower Gd-IgA1 staining intensity [14, 16] in cases of lupus nephritis accompanied by significant IgA deposition.

Lupus nephritis and IgA nephropathy can be readily diagnosed and distinguished based on pathologic features on renal biopsy when the classic patterns are present. However, unusual patterns such as dominant glomerular IgA deposition can become a diagnostic challenge as to whether it represents primary IgA nephropathy or atypical lupus nephritis. Here we report immunostaining of KM-55 successfully distinguishes lupus nephritis from primary IgA nephropathy in a diagnostic dilemma.

Case Presentation

Clinical History and Laboratory Data

An 11-year-old Vietnamese girl with no significant past history presented with malar rash on cheeks and patchy alopecia on the scalp. There is no history of lupus or kidney disease on the mother's side of the family. Family history of father's side is unknown. The initial pertinent laboratory results showed a serum Cr of 0.43 mg/dL. Urinalysis (UA) revealed 3 RBCs/HPF without proteinuria. Serological testing was positive for ANA (1:1,280; reference range <1:80) with speckled pattern and double-stranded DNA antibody (dsDNA) (1:320; reference range <1:10). Complement levels were C3 66 (reference range 80–160) and C4 14 (reference range 13–44). The patient was referred to the pediatric rheumatology clinic. Clinical and laboratory findings fulfilled the diagnostic criteria for SLE [17], and the patient was diagnosed with SLE without clinically apparent renal involvement. The patient was started with oral prednisone (20 mg a.m. and 15 mg p.m.) and hydroxychloroquine (200 mg Monday–Friday and 300 mg Saturday–Sunday). At 8-week follow-up, the serum Cr was 0.53 mg/dL, UA revealed 50 RBCs/HPF and 100 mg/dL proteinuria, and serologic testing was positive for dsDNA (1:80) with normalized C3 (105, reference range 83–193) and C4 (23.4, reference range 15–57) complement levels. The patient was referred to the pediatric nephrology clinic for the evaluation of worsening hematuria and new-onset proteinuria despite improved dsDNA and complements. At this visit, the patient was feeling well overall. She described multiple symptoms that begun after starting steroids/hydroxychloroquine including swelling/closeness of her cheeks, tremor in both her hands, weight gain, and hair loss. She denied gross hematuria, dysuria, joint pain, oral sores, or swelling in her legs and arms. Her laboratory results at 12 weeks after initial presentation were serum Cr 0.38 mg/dL, 3 RBCs/HPF, protein:Cr ratio 0.75 g/g, C3 124 (reference range 97–196), and C4 31 (reference range 11–37). To better define the renal involvement, a kidney biopsy was performed.

Kidney Biopsy Findings and Diagnostic Dilemma

The biopsy showed 1 needle core of renal cortical tissue with attached capsule and 1 needle core of renal cortico-medullary tissue. On serial sections, 26 glomeruli were present, none of which were globally sclerotic. Slightly <50% of the glomeruli showed segmental moderate mesangial hypercellularity with associated mesangial expansion (Fig. 1a). Glomerular basement membranes were smooth and single contoured. No endocapillary hypercellularity, "wire loop" lesions, necrosis, crescents, or segmental sclerosis were noted. Tubules, interstitium, and vasculature were unremarkable. Immunofluorescence microscopy revealed segmental, granular mesangial staining for IgG (2+), IgA (3+), IgM (1+), C1q (trace), C3 (trace), and kappa (3+) and lambda (3+) light chains (Fig. 1b–h). The staining for albumin and fibrinogen was negative. No extraglomerular staining was seen. Ultrastructurally, glomerular basement membranes had a normal trilaminar structure and a mean thickness within normal range. There were many electron-dense deposits in mesangial regions. No substructure was identified. No evident subendothelial, subepithelial, or intramembranous deposits were noted. There was <50% podocyte foot process effacement. Endothelial cells showed mostly preserved fenestration. No tubuloreticular inclusions were seen in endothelial cells.

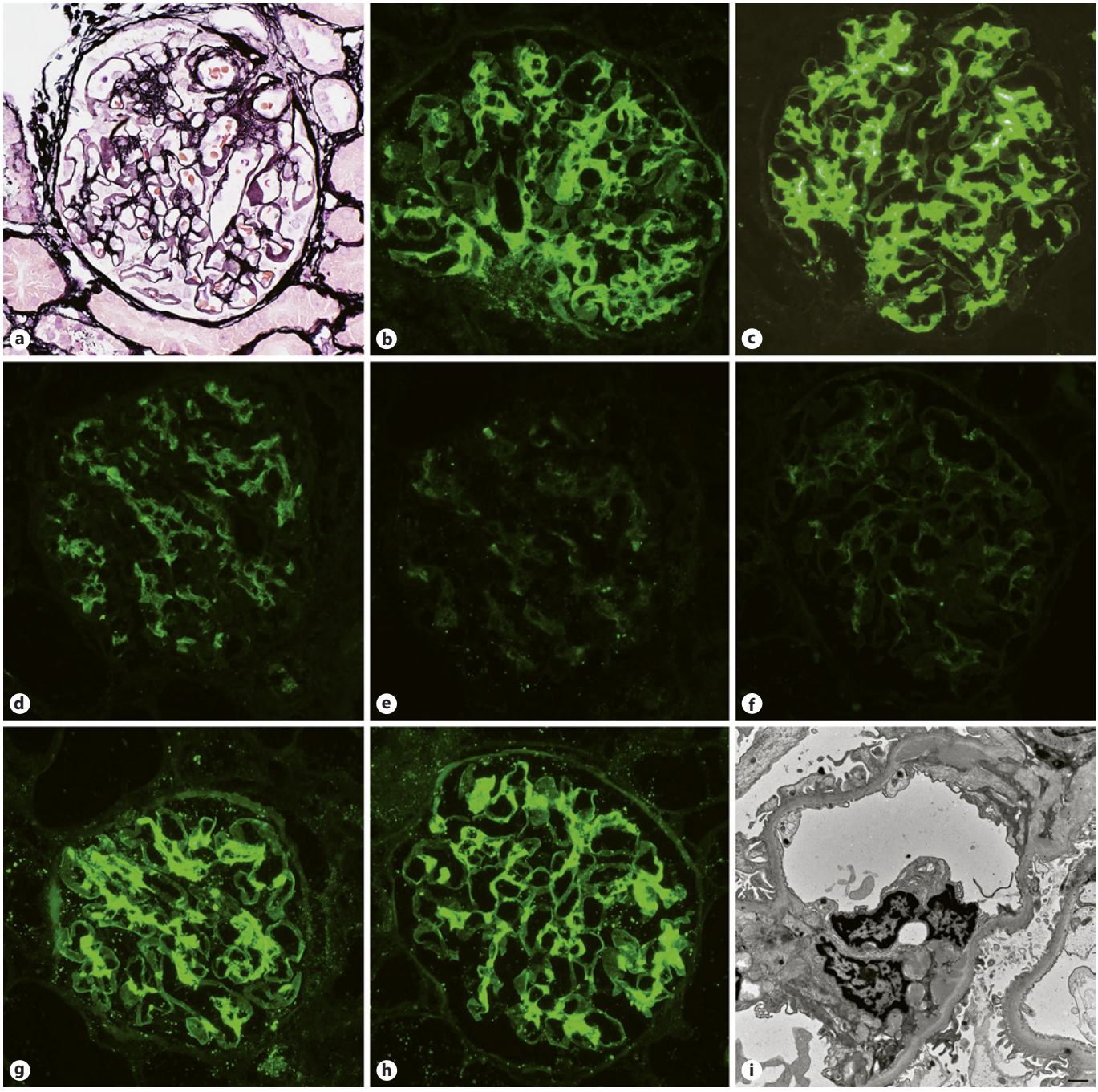

Fig. 1. A glomerulus with segmental mesangial hypercellularity and associated mesangial expansion (**a**, silver stain). Immunofluorescence microscopy shows segmental granular mesangial staining for IgG (2+), IgA (3+), IgM (1+), C1q (trace), C3 (trace), and kappa (3+) and lambda (3+) light chains (**b–h**, respectively). **i** Electron-dense deposits are seen in mesangium. Original magnification ×400 in **a–h** and ×8,000 in **g** (scale bar, 1 μm).

The differential diagnoses included IgA nephropathy (Oxford classification: M0E0S0T0C0) or class II lupus nephritis with atypical immunofluorescence, although the glomerular IgA dominance with weak C1q and C3 staining favored the diagnosis of IgA nephropathy.

Gd-IgA1 Immunohistochemical Staining on Kidney Biopsy

Formalin-fixed paraffin-embedded kidney tissue was stained with anti-human Gd-IgA1 (KM-55) rat IgG monoclonal antibody (Immuno-Biological Laboratories Co., Ltd., at a concentration of 100 μg/mL, Table 1) with appropriate positive control (IgA ne-

Table 1. KM-55 paraffin immunofluorescence procedure

Deparaffinize: xylene for 15 min (×2), ethanol 100% for 5 min (×2), 95% for 5 min, 80% for 5 min, and 70% for 5 min
Rinse in tap water for 5 min
Incubate with subtilisin A solution* for 2 h at room temperature
Rinse with TBST for 5 min (×3)
Incubate with blocking buffer# for 1 h at room temperature
Incubate with KM-55 (100 μg/mL) overnight at 4 C
Rinse with TBST for 5 min (×3)
Incubate with secondary antibody (Alexa 488-conjugated goat anti-rat) for 45 min at room temperature
Rinse with TBST for 5 min (×3)
Mount in Vectashield Aqueous Mounting Media

* Subtilisin A solution: 0.05% (w/v) subtilisin A (Sigma, P5380) in 5 mM Tris-HCI (pH = 7.6). # Blocking buffer: 10 mL 1 × PBS with 30 μL (0.3%, v/v) IGEPAL = CA630, and BSA 0.1 g (1%, w/v).

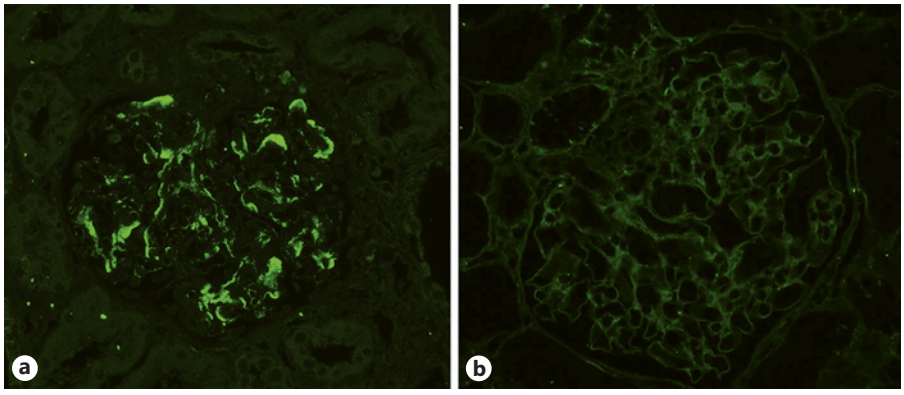

Fig. 2. a In appropriate control (IgA nephropathy), the representative glomerulus shows global, granular, mesangial staining of KM-55, in a similar pattern and intensity to IgA. **b** No glomerular KM-55 immunofluorescence staining is detected in our patient's kidney biopsy. Original magnification ×400 in both. IgA, immunoglobulin A.

phropathy; Fig. 2a). No detectable glomerular KM-55 staining was seen in this patient's kidney biopsy (Fig. 2b).

Clinical Follow-up

The patient was treated with immunosuppression consisting of prednisone and mycophenolate mofetil (250 mg twice daily by mouth) in addition to lisinopril (2.5 mg daily by mouth). At 3-month follow-up, the patient was doing well and denied gross hematuria, joint pains, swelling, or rashes. Serum Cr was 0.51 mg/dL. UA showed 1 RBC/HPF without proteinuria.

Discussion and Conclusions

Although the spectrum of renal involvement in SLE is heterogenous, the histologic diagnosis of lupus nephritis is usually straight-forward. The most characteristic feature of lupus nephritis is IgG-dominant "full house" with intense C1q immunofluorescence staining. The deposits range from small, discrete, mesangial electron-dense deposits (in lupus nephritis class I or II) to copious mesangial deposits with variable numbers and extent of subendothelial, subepithelial, and intramembranous deposits, with or without extraglomerular deposits.

IgA nephropathy is characterized by dominant/codominant IgA deposition in glomerular mesangium. Glomerular IgG, IgM, and C1q deposition, in the same distribution as IgA, is observed in approximately 45, 55, and 10% of patients with IgA nephropathy, respectively [18]. A recent study shows, out of 627 IgA nephropathy renal biopsies, glomerular IgG deposition is detected in 200 biopsies, 178 mild (grade 1), and 22 moderate to marked (grade 2–3) [19].

Approximately 0.3% (9 out of 251) ESRD in SLE patients is attributed to nonlupus nephritis [20]. IgA ne-

Table 2. Summary of KM-55 paraffin immunofluorescence staining in primary IgA nephropathy and lupus nephritis

Reference	IgA nephropathy positive, *n*/*N* (%)	Median intensity	Intensity range	Lupus nephritispositive, *n*/*N* (%)	Median intensity	Intensity range
Suzuki et al. [13]	48/48 (100)	ns	ns	0/7 (0)	0	0
Wang et al. [15]	4/4 (100)	ns	ns	0/ns (0)	0	0
Cassol et al. [14]	44/44 (100)	2+	0.5+ to 3+	3/8 (37.5)	0	0–1+
Zhao etal. [16]	40/40 (100)	3+	0.5+ to 4+	11/11 (100)	1+	0.5+ to 4+

The intensity scale was 0–3+ and 0–4+ for references Cassol et al. [14] and Zhao etal. [16], respectively. All lupus nephritis cases showed significant IgA deposition. IgA, immunoglobulin A; ns, not specified.

phropathy in SLE patients has been previously described in a total of 10 patients from 8 reports [5–12]. One patient had 2 sequential kidney biopsies, lupus nephritis class II on the initial biopsy, and IgA nephropathy on the follow-up biopsy after treatment [9]. Another IgA nephropathy in SLE patient showed intense global granular glomerular IgA and C3 deposition with absence of C1q staining, however, subendothelial, subepithelial, and intramembranous electron-dense deposits were also present [12]. The diagnosis of IgA nephropathy were rendered for all the reported cases; however, the pathologic features were not classic for IgA nephropathy at least in some of these cases as mentioned above.

Gd-IgA1 is a key element in the pathogenesis of primary IgA nephropathy. Gd-IgA1 is elevated in serum, and glomerular Gd-IgA1 deposits, detected by immunofluorescent staining of KM-55, are consistently identified in the mesangium of IgA nephropathy. Immunofluorescence staining of KM-55 has been examined in lupus nephritis in several studies since the introduction of this antibody (Table 2) [13–16]. In the studies of Suzuki et al. [13] and Wang et al. [15], all lupus nephritis cases with significant IgA deposition are negative for KM-55 (0/23, combined). On a scale of 0–3+, weak KM-55 staining was observed in 3 out of 8 lupus nephritis cases (0.5+ in 2 cases and 1+ in 1 case) with a median intensity of 0, in comparison to a median intensity of 2+ for primary IgA nephropathy [14]. Variable but significantly less intense KM-55 staining (median 1+) was seen in all 11 lupus nephritis cases in the study of Zhao et al. [16], in comparison to a median intensity of 3+ in primary IgA nephropathy on a scale of 0–4+. Overall, at least a 2 grade less staining intensity of KM-55 was observed in lupus nephritis comparing with primary IgA nephropathy, when KM-55 staining is not negative (Table 2). Thus, we performed KM-55 staining on this challenging kidney biopsy to differentiate between IgA nephropathy and lupus nephritis. The absence of KM-55 staining excludes IgA nephropathy and supports a diagnosis of lupus nephritis with atypical immunofluorescence in this patient.

The renal biopsy findings in our SLE patient present a diagnostic dilemma between IgA nephropathy and lupus nephritis with atypical immunofluorescence, with focal mesangial hypercellularity and IgA dominant mesangial deposits, accompanied by various amount of IgM, IgG, C3, and C1q staining, in the absence of strong C1q or C3 staining, extraglomerular deposits, tissue ANA, and endothelial tubuloreticular inclusions. It is noted the patient has been on steroids and hydroxychloroquine for 12 weeks prior to the kidney biopsy. It is unknown whether the glomerular dominance of IgA and the weak C1q and C3 staining might be related to the treatment of SLE prior to kidney biopsy.

Accurate interpretation of renal biopsy in SLE patients is critical for the optimal management of these patients. Immunostaining for KM-55 may have unique diagnostic utility in challenging kidney biopsy of SLE patient with features suggestive of IgA nephropathy.

Statement of Ethics

All procedures in this study were performed in accordance with the ethical standards of the University of Minnesota; consent for publication: written informed consent was obtained from the patient's guardian for publication of this case report and the images in it.

Conflict of Interest Statement

All the authors declared no competing interests.

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Author Contributions

L.B. and Y.K. wrote the manuscript. A.M.K. treated the patient. B.Y. performed the KM-55 immunostaining. All authors read and approved the final manuscript.

Availability of Data and Material

All data and material were presented in this manuscript.

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