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Renal tubular lysozyme in tubulointerstitial diseases and renal sarcoidosis

Satoru Sanada, Mitsuhiro Sato
Division of Nephrology, Japan Community Health Care Organization Sendai Hospital, Sendai, Miyagi, Japan

To the editor,

Tubulointerstitial diseases are one of the common features of kidney diseases. Histological characteristics include infiltration of inflammatory cells, tubular atrophy, and interstitial fibrosis. Causes of tubular damage vary, including drugs, systemic inflammatory diseases, genetic disorders, infections, ischemia, toxins, and obstructive uropathy. To understand tubulointerstitial diseases, types of infiltration of inflammatory cells to interstitial areas can be classified into three categories; i) tubulitis caused by inflammation, so-called tubulointerstitial nephritis (TIN), ii) reaction to tubular atrophy caused by non-immune-mediated mechanisms such as ischemia, and iii) proliferation of inflammatory cells represented by posttransplant lymphoproliferative disorders (1). However, differentiating based solely on specimen information is sometimes challenging, and clinical presentation with laboratory data provides clues for the diagnosis. Lysozyme-induced nephropathy is one unique cause of tubulointerstitial disease, which is a rare comorbidity of chronic myelomonocytic leukemia (2). Massive amounts of lysozyme secreted from abnormal monocytes are filtrated through the glomeruli and flow into renal tubules. Lysozyme in the raw urine is absorbed into renal proximal tubules via its receptors of megalin (3). Over-accumulation of lysozyme damages proximal tubular cells leading to tubulointerstitial injury. In this condition, lysozyme immunohistochemistry demonstrates distinct positive staining in proximal tubular cells (2). Distinctive renal involvement in sarcoidosis is TIN; however, the underlying mechanisms remain unclear. Granuloma formation is a typical finding in renal sarcoidosis, but its detection rate is low (4). Although hypercalcemia is believed to contribute to kidney injury, the infiltration of inflammatory cells in the tubulointerstitial areas suggests that other mechanisms, beyond hypercalcemia, should be considered. Previously, we noted that TIN caused by sarcoidosis exhibits positive lysozyme staining in the same pattern as lysozyme-induced nephropathy shown in chronic myelomonocytic leukemia (5). Elevated levels of plasma lysozyme from activated monocytes play a crucial role in the inflammatory processes associated with sarcoidosis (6). In this report, we assess the characteristics of lysozyme staining using 96 kidney biopsy samples from patients with tubulointerstitial diseases to confirm the effectiveness of lysozyme staining for diagnosing renal sarcoidosis.

Among 1821 kidney biopsy samples collected from 2016 to 2023 at Japan Community Health Care Organization Sendai Hospital, 96 were identified as tubulointerstitial disease (Figure 1).

These cases were stained with lysozyme using immunohistochemistry. Immunoperoxidase staining was conducted with an anti-lysozyme antibody (dilution 1:3000; BosterBio, Pleasanton, CA, USA) on paraffin-embedded tissue samples. Each specimen was evaluated and classified as either lysozyme positive or negative on renal tubules (Figure 2).

If lysozyme staining was observed in even a portion of the tubular lesions, the specimen was deemed lysozyme positive. Tubulointerstitial diseases of unknown etiology were classified as idiopathic. The study protocol received approval from the Ethics

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Correspondence: Satoru Sanada, MD, PhD
Address: 2-1-1, Murasakiyama, Izumi, Sendai, Miyagi, Japan
E-mail: satsanada@sendai-kidney.jp
ORCID: 0000-0002-7063-424X

Committee of the Japan Community Health Care Organization Sendai Hospital (protocol number: 2025-05).

The diagnoses of 96 kidney specimens are detailed in Table 1. Lysozyme positive staining was observed in proximal tubular cells. In contrast, distal tubules, collecting ducts, and glomeruli were all negative for lysozyme in every specimen. Out of 96 samples, 24 demonstrated positive lysozyme staining, including 13 cases of sarcoidosis-associated TIN, one case of CMML-associated lysozyme-

induced nephropathy, one case of inflammatory cell infiltration in B-cell lymphoma, one case of ulcerative colitis-associated TIN, three cases of drug-associated TIN, and five cases of idiopathic TIN. Notably, strong immunohistochemical lysozyme positivity was observed in the CMML-associated TIN, where the serum lysozyme concentration was significantly elevated at 166.5 μg/mL. The intensity of lysozyme positivity in other etiologies was comparatively lower than that observed in the CMML case. Table 2 presents the characteristics of 13 cases of renal sarcoidosis. All cases had elevated serum lysozyme concentrations, with an average of 33.9 μg/mL. No lysozyme-positive staining was observed in 72 samples of the following conditions: ischemic tubulointerstitial diseases, IgG4-related disease, Sjögren's syndrome, hereditary cystic diseases, aristolochic acid-related nephritis, cast nephropathy in multiple myeloma, light-chain deposition disease, and obstructive uropathy. The sensitivity and specificity of lysozyme immunohistochemistry for diagnosing renal sarcoidosis from TIN were 100% and 86.7%, respectively.

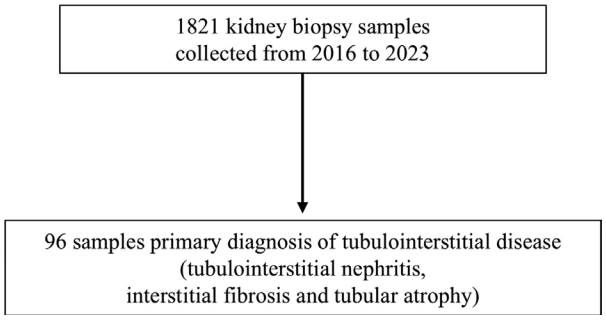

Figure 1. Study flowchart.

The details of 18 cases of drug-induced TIN are presented in Table 3. Among these, two cases related to 5-aminosalicylic acid (5-ASA) treatment for ulcerative colitis showed positive lysozyme staining. In contrast, TIN associated with treatment for Crohn's disease using 5-ASA exhibited negative lysozyme staining. Additionally, one case of immune checkpoint inhibitor combined with a proton pump inhibitor for lung adenocarcinoma showed positive lysozyme staining, however, the other four cases of immune checkpoint inhibitor with proton pump inhibitor-associated TIN, which included three instances of lung adenocarcinoma and one case of gingival cancer, were negative for lysozyme staining.

This study provides evidence of the effectiveness of lysozyme immunohistochemistry against tubulointerstitial diseases in narrowing down the differential diagnosis or detecting underdiagnosed diseases. Notably, renal sarcoidosis can be detected

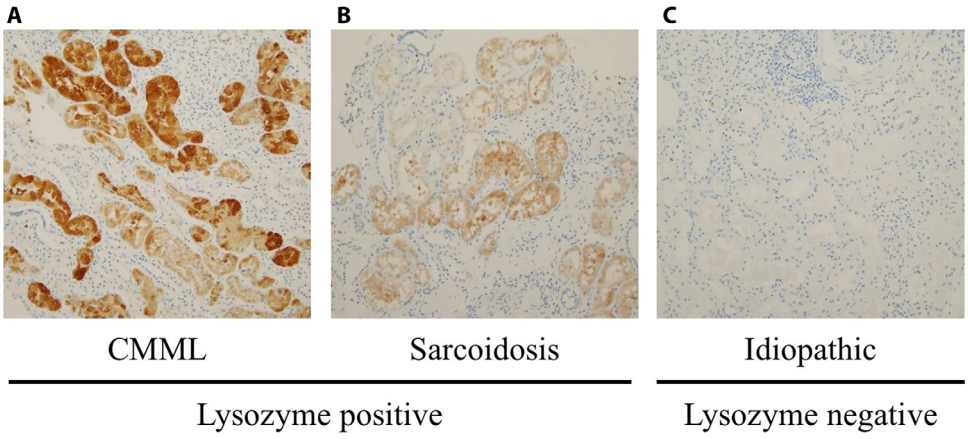

Figure 2. Lysozyme immunohistochemistry. A) renal biopsy sample from chronic myelomonocytic leukemia. B) sample from renal sarcoidosis. C) sample from idiopathic tubulointerstitial nephritis.

Table 1. Lysozyme positivity in relation to underlying diseases.

Diseases	Total (n=96)	Positive (n=24)	Negative (n=72)	Positive rate (%)
Sarcoidosis	13	13	0	100
CMML	1	1	0	100
B cell lymphoma	1	1	0	100
Ulcerative colitis	1	1	0	100
Drug	18	3	15	16
Idiopathic	31	5	26	16
Ischemia	18	0	18	0
IgG4 RD	4	0	4	0
Sjogren syndrome	3	0	3	0
ADTKD	2	0	2	0
Aristolochic Acid	1	0	1	0
Cast nephropathy	1	0	1	0
LCDD	1	0	1	0
Obstructive uropathy	1	0	1	0

The number of cases tested and the percentage of positivity for lysozyme immunohistochemistry based on various underlying tubulointerstitial diseases. *Abbreviations:* CMML; chronic myelomonocytic leukemia, IgG4 RD; IgG4-related disease, ADTKD; autosomal dominant tubulointerstitial kidney disease, LCDD; light chain deposition disease.

Table 2. Characteristics of 13 cases of renal sarcoidosis.

Age (y)	Gender (M/F)	Cr (mg/dL)	LYZ (μg/mL)	ACE (U/L)	sIL2R (U/mL)	Urine Protein (g/gCr)	Urine Red blood cell (/HPF)	u-β2MG (μg/L)	Organs involved	Tissue granuloma
71	F	1.35	14.4	18.6	NA	0.1	<1	3869	Skin, Eye, Heart, Lymph	Skin
79	F	2.33	26.7	20.9	870	0.2	<1	940	Eye, Lymph	-
76	F	1.83	29.2	9.9	1500	0.3	<1	4091	Lung	Lung
44	F	1.14	13.9	7.4	NA	0.3	<1	45326	Heart	-
51	F	1.95	17.7	10.0	784	0.2	<1	1198	Heart	-
41	F	7.76	63.0	16.8	NA	0.6	<1	9586	Eye	Kidney
74	F	1.17	55.8	41.7	2570	0.8	<1	20589	Eye, Lymph	Kidney
66	F	2.22	57.4	24.4	2870	0.7	<1	49814	Lung, Lymph	Kidney
55	M	2.59	24.7	20.9	1410	0.2	<1	20390	Lung, Lymph	-
71	F	1.28	56.3	14.4	NA	0.9	<1	45101	-	-
63	M	1.65	34.5	24.4	NA	1.2	<1	192908	Liver	Kidney
58	F	1.34	24.6	21.4	916	0.1	<1	10146	Lung, Skin	-
51	F	1.66	23.0	9.3	1360	0.1	<1	3962	-	Kidney

Abbreviations: Cr; serum creatinine, LYZ; serum lysozyme, ACE; serum angiotensin converting enzyme, sIL2R; serum soluble interleukin-2 receptor, u-β2MG; urinary β2-microglobulin, NA; not applicable.

Table 3. Lysozyme positivity in drug-induced tubulointerstitial diseases.

Drugs	Total (n=18)	Positive (n=3)	Negative (n=15)	Positive rate (%)
5-ASA to UC	2	2	0	100
5-ASA to Crohn	2	0	2	0
ICI + PPI	5	1	4	20
Antibiotics	3	0	3	0
Anti-EGFR	1	0	1	0
Diltiazem	1	0	1	0
Puberulic acid	1	0	1	0
Sodium phosphate	1	0	1	0
Lithium	1	0	1	0
Unknown	1	0	1	0

The list of 18 cases of drug-induced tubulointerstitial nephritis, with the percentage of lysozyme positivity. *Abbreviations:* 5-ASA; 5-aminosalicylic acid, UC; ulcerative colitis, Crohn; Crohn's disease, ICI; Immune checkpoint inhibitor, PPI; proton pump inhibitor, EGFR; epidermal growth factor receptor.

with 100% accuracy, as all 13 cases examined showed positive lysozyme staining. This finding suggests that lysozyme immunohistochemistry can be valuable for diagnosing sarcoidosis-associated TIN. However, it is important to carefully consider whether the positive lysozyme staining observed in proximal tubules indicates lysozyme-induced nephropathy or if elevated plasma lysozyme levels lead to positive staining without causing harm to the tubular cells. Interestingly, all three ulcerative colitis cases showed lysozyme positive regardless of treatment using 5-ASA. TIN is a rare renal complication of irritable bowel diseased of ulcerative colitis and Crohn's disease (7). In addition, their treatment option of 5-ASA induces TIN as well (8). These factors make it difficult to understand the pathogenesis of TIN associated with irritable bowel diseases. When comparing the two conditions, it is known that plasma lysozyme levels are elevated in ulcerative colitis, while they remain within normal limits in Crohn's disease (9). Our findings of lysozyme positivity in ulcerative colitis, irrespective of treatment with 5-ASA, may suggest that lysozyme staining does not accurately reflect the pathogenesis of TIN. This is due to the possibility that the mechanisms behind the development of TIN could differ based on disease activity or medication. Positive lysozyme staining of proximal tubular cells may result from high plasma lysozyme concentrations and increased absorption in proximal tubules. The upcoming question is: how much lysozyme is required to induce proximal tubular damage and lysozyme-induced nephropathy? Hsu et al. reported interesting data showing that in a leukemia rat model, excessive plasma lysozyme levels led to kidney injury, characterized by positive lysozyme staining in the proximal tubules, similar to what is seen in lysozyme-induced nephropathy (10). However, when lysozyme was injected into normal rats, it did not induce kidney damage, indicating that lysozyme alone cannot cause lysozyme-induced nephropathy. Furthermore, Haas et al. demonstrated in a rat experimental model that the renal tubular absorption rate of the non-steroidal anti-inflammatory drug naproxen is increased when it is conjugated with lysozyme. This finding suggests that lysozyme plays a specific role in the reabsorption process in the proximal tubules by recruiting other ligands, which could be linked to kidney injury (11). Megaline, a receptor for lysozyme, is a large endocytosis receptor that can interact with various ligands, including natural products such as vitamins and non-natural compounds such as antibiotics (12). Multiple pockets on megalin enable interaction with multiple ligands (13). While the mechanisms of megalin-dependent internalization have been gradually elucidated, the ligand-specific actions are not yet fully understood. Together, the pathogenesis of lysozyme-induced nephropathy may require more information to clarify the underlying mechanisms. Nonetheless, even if lysozyme positivity may not reveal the cause of tubular damage,

it is evident that lysozyme immunohistochemistry is a valuable tool for diagnosing renal sarcoidosis. Furthermore, lysozyme immunohistochemistry may offer additional information on tubulointerstitial diseases, particularly those with unknown etiology.

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Conflict of Interest: Each author declares that he has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article.

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