Pathological value of lysozyme staining for renal sarcoidosis

Satoru Sanada, Shohei Yoda and Toshinobu Sato
Division of Nephrology, Japan Community Health Care Organization Sendai Hospital, Sendai, Miyagi, Japan

Correspondence to: Satoru Sanada; E-mail: satsanada@sendai-kidney.jp

Sarcoidosis is a systemic inflammatory disease of unknown etiology. Besides kidneys, multiple organs including the lungs, skin, heart, eyes and lymph nodes are affected with or without granulomatous formations. Diagnosis of sarcoidosis is often difficult because of its few clinical symptoms and a low detection rate of granulomas in the tissue. Even the pathological finding of granulomas cannot directly connect to the diagnosis, as it can be found in other inflammatory diseases. Kidney biopsy specimens of renal sarcoidosis show interstitial fibrosis and tubular atrophy with mononuclear cell infiltrations. In most cases, these findings cannot provide adequate diagnostic information because the etiology of tubulointerstitial nephritis (TIN) is diverse: infections, drugs, toxins, autoimmune diseases and other factors induce tubular damage as well. Among the pathogenesis of TIN, sarcoidosis could be one of the most underdiagnosed afflictions because of its variety of symptoms and its slow progression of the disease. Therefore, reliable diagnostic tools are required.

Some laboratory measurements that include high serum calcium, angiotensin-converting enzyme and lysozyme levels are helpful for approaching the diagnosis [1]. Lysozyme is a glycoside hydrolase that catalyzes peptidoglycans on bacterial cell walls resulting in antibacterial activity [2]. In sarcoidosis, monocytes/macrophages are known to be a key effector of disease activity, and lysozyme is produced by these cells [3, 4]. Once in the serum, circulating lysozyme is filtrated in the glomeruli and reabsorbed in the proximal tubules, where lysozyme is digested within lysosomes [5]. It is well known that chronic myelomonocytic leukemia (CMML), a hematologic disorder of clonal monocytes, shows a high serum lysozyme level that induces severe TIN with swollen proximal tubules containing a large number of lysosomes and a strongly positive lysozyme [6, 7]. In

Nephrol Dial Transplant (2020) 35: 1638–1641
doi: 10.1093/ndt/gfaa070
Advance Access publication 21 May 2020

15. Lei S, Jiang F, Su W et al. Clinical characteristics and outcomes of patients undergoing surgeries during the incubation period of COVID-19 infection. EClinicalMedicine 2020; 21: 100331
16. Cai Q, Huang D, Ou P et al. COVID-19 in a designated infectious diseases hospital outside Hubei Province, China. Allergy 2020; 75: 1742–1752
17. Pei G, Zhang Z, Peng J et al. Renal involvement and early prognosis in patients with COVID-19 Pneumonia. J Am Soc Nephrol 2020; 31: 1157–1165
18. Hoffmann M, Kleine-Weber H, Schroeder S et al. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. Cell 2020; 181: 271–280.e8
19. Zhang H, Penninger JM, Li Y et al. Angiotensin-converting enzyme 2 (ACE2) as a SARS-CoV-2 receptor: molecular mechanisms and potential therapeutic target. Intensive Care Med 2020; 46: 586–590
20. Pan WX, Xu D, Zhang H et al. Identification of a potential mechanism of acute kidney injury during the COVID-19 outbreak: a study based on single-cell transcriptome analysis. Intensive Care Med 2020; 46: 1114–1116
21. Puéllès VG, Lutgethmann M, Lindenmeyer MT et al. Multiorgan and renal tropism of SARS-CoV-2. N Engl J Med 2020; 383: 590–592
22. Palmieri L, Vanacore N, Donfrancesco C et al. Clinical characteristics of hospitalized individuals dying with COVID-19 by age group in Italy. J Gerontol A Biol Sci Med Sci 2020; doi:10.1093/gerona/glaa146
23. Hirsch JS, Ng JH, Ross DW et al. Acute kidney injury in patients hospitalized with COVID-19. Kidney Int 2020; 98: 209–218
24. Cheng Y, Luo R, Wang K et al. Kidney disease is associated with in-hospital death of patients with COVID-19. Kidney Int 2020; 97: 829–838
25. Zhang W, Du RH, Li B et al. Molecular and serological investigation of 2019-nCoV infected patients: implication of multiple shedding routes. Emerg Microbes Infect 2020; 9: 386–389
26. Zhang Y, Fan J, Yu F et al. Viral load dynamics and disease severity in patients infected with SARS-CoV-2 in Zhejiang province, China, January-March 2020: retrospective cohort study. BMJ 2020; 369: m1443
27. Dreher M, Kersten A, Bickenbach J et al. The characteristics of 50 hospitalized COVID-19 patients with and without ARDS. Dtsch Arztebl Int 2020; 117: 271–278
28. Chen X, Zhao B, Qu Y et al. Detectable serum SARS-CoV-2 viral load (RNAemia) is closely correlated with drastically elevated interleukin 6 (IL-6) level in critically ill COVID-19 patients. Clin Infect Dis 2020; doi: 10.1093/cid/ciaa449
29. Chan JF, Yuan S, Kok KH et al. A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. Lancet 2020; 395: 514–523
30. Kim JY, Ko JH, Kim Y et al. Viral load kinetics of SARS-CoV-2 infection in first two patients in Korea. J Korean Med Sci 2020; 35: e86
31. Ding Y, He L, Zhang Q et al. Organ distribution of severe acute respiratory syndrome (SARS) associated coronavirus (SARS-CoV) in SARS patients: implications for pathogenesis and virus transmission pathways. J Pathol 2004; 203: 622–630

© The Author(s) 2020. Published by Oxford University Press on behalf of ERA-EDTA.
This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/4.0/), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited. For commercial re-use, please contact journals.permissions@oup.com
CMML, accumulated lysozyme in the proximal tubules induces tubular damage that is recognized as lysozyme-induced nephropathy [8]. Based on these findings, we hypothesized that TIN in sarcoidosis could be the same mechanism as is found in CMML kidney injury. Excessive lysozyme reaching proximal tubular cells may play an essential role in the pathogenesis of tubular injury in sarcoidosis.

Forty-three kidney biopsy specimens diagnosed as TIN collected at the Japan Health Care Organization Sendai Hospital were stained with antilysozyme antibody. The etiology of TIN included sarcoidosis (n = 6), CMML (n = 1), idiopathic (n = 9), drug-induced (n = 12), ischemic kidney injury with nonspecific infiltrate in the fibrotic areas (n = 9), tuberculosis (n = 1), light chain deposition disease (n = 1), Sjögren’s syndrome (n = 1), immunoglobulin G4 (IgG4)-related disease (n = 1), cast nephropathy (n = 1) and arilostolic acid–related TIN (n = 1) (Figure 1A). Among them, 10 cases were lysozyme positive in proximal tubular cells: 6 cases of sarcoidosis [6/6 (100%), 1 case of CMML [1/1 (100%)] and 3 cases of idiopathic TIN [upper panels of idiopathic TIN in Figure 1A; 3/9 (33%)]. Lysozyme stains were blunted in the other TIN specimens. In CMML, a strong lysozyme stain in the proximal tubules was observed that is compatible with previous studies in both humans and rats [7, 9]. Although staining intensity was not as strong as CMML, all

---

**FIGURE 1:** (A) Kidney specimens of 43 TIN patients stained with antilysozyme antibody. Magnification ×100. (B) Electron microscopy images of tubular epithelial cells in CMML, sarcoidosis and IgG4-related disease. Magnification ×3000.
Six sarcoidosis specimens showed mild to moderate lysozyme stains in the proximal tubules in the same manner as CMML.

Serum lysozyme levels were measured in 39 of 43 participants (Figure 2). Patients with positive lysozyme stains (black circle) showed higher serum lysozyme concentrations compared with those with negative stains (gray circle) ($P < 0.005$), suggesting that circulating lysozyme level could associate with renal tubular lysozyme. In sarcoidosis, the serum lysozyme level increased slightly but not as much as that of CMML. These results may explain the difference in the intensity of lysozyme staining in kidney tubular cells; strong lysozyme staining in CMML and weak in sarcoidosis could be the result of the amount of lysozyme flowing into the proximal tubules. In electron microscopy, an increased number of enlarged electron-dense lysosomes were present in the cytoplasm of proximal tubules in CMML and sarcoidosis; however, these changes were absent in IgG4-related renal disease (Figure 1B). Although these changes are not specific for lysozyme-induced tubular injury, enlarged proximal tubular lysosomes are common in CMML nephropathy [6, 8]. It is known that proximal tubular cells absorb lysozyme via endocytosis to degrade in lysosomes [10, 11]. We think that lysosomes in the proximal tubular cells were processing overloaded lysozyme both in CMML and sarcoidosis. Lysozyme-induced nephropathy is the cause of severe interstitial nephritis in CMML, but the same mechanism may explain the pathogenesis of renal sarcoidosis despite mild to moderate severity compared with CMML. The mechanisms of renal impairment in sarcoidosis have been thought to result from hypercalcemia or granulomatosis [12]; however, our findings suggest that lysozyme-induced tubular damage could be a main cause of TIN in sarcoidosis.

In this study, three idiopathic TIN cases were lysozyme positive in spite of lacking characteristic symptoms of sarcoidosis. The etiology of these three cases is unknown, suggesting that lysozyme staining is not specific for renal sarcoidosis. However, it cannot be denied that these three patients were in an early or stable period of renal sarcoidosis because two of the three cases demonstrated slightly elevated serum lysozyme concentrations (Figure 2). The severity and activity of disease are important for assessing pulmonary sarcoidosis, of which pathological findings vary according to stages, whereas there is no clear strategy to determine the severity of renal sarcoidosis. Lysozyme stains were positive in these idiopathic TIN cases, but their intensities were faint. We expected that mild lysozyme stain may detect potential renal sarcoidosis.

Active tuberculosis is known to show increased serum lysozyme levels and TIN could be observed as a renal involvement [13, 14]. In this study, lysozyme stain was negative in one case of TIN with tuberculosis. However, we do not have serum lysozyme data for this patient. If an excessive amount of lysozyme acts as an antibacterial agent against tuberculosis, lysozyme-induced tubular injury could be a cause of tuberculosis-associated TIN. Further studies are necessary to conclude whether lysozyme staining could be helpful for diagnosing tuberculosis-associated TIN.

A limitation of this study is the small sample number. Lysozyme staining is positive in all six sarcoidosis cases, but we cannot conclude that renal sarcoidosis always shows the same result. Furthermore, being lysozyme positive is not specific for sarcoidosis, as we have shown positive cases in CMML and idiopathic TIN, indicating that being lysozyme positive is not directly connected to renal sarcoidosis. However, given that most TIN cases revealed negative lysozyme staining in this study, we believe our approach could be effective to extract renal sarcoidosis from pathologically diagnosed TIN diseases.

In conclusion, we demonstrated the effectiveness of lysozyme immunohistochemistry for diagnosing renal sarcoidosis. This method can help with the detection of underdiagnosed sarcoidosis.

**ACKNOWLEDGEMENTS**

The authors would like to thank Hiroshi Kitamura and Susumu Onodera for their excellent technical assistance.
FUNDING

The study was funded by a Miyagi Association of Kidney Disease Patients research grant.

CONFLICT OF INTEREST STATEMENT

None declared.

REFERENCES

1. Tomita H, Sato S, Matsuda R et al. Serum lysozyme levels and clinical features of sarcoidosis. Lung 1999; 177: 161–167
2. Hansen NE, Karle H, Andersen V et al. Lysozyme turnover in man. J Clin Invest 1972; 51: 1146–1155
3. Prokop S, Heppner FL, Goebel HH et al. M2 polarized macrophages and giant cells contribute to myofibrosis in neuromuscular sarcoidosis. Am J Pathol 2011; 178: 1279–1286
4. Bergantini L, Bianchi F, Cameli P et al. Prognostic biomarkers of sarcoidosis: a comparative study of serum chitotriosidase, ACE, lysozyme, and KL-6. Dis Markers 2019; 2019: 1–7
5. Christensen EI, Maunsbach AB. Intralysosomal digestion of lysozyme in renal proximal tubule cells. Kidney Int 1974; 6: 396–407
6. Patel TV, Rennke HG, Sloan JM et al. A forgotten cause of kidney injury in chronic myelomonocytic leukemia. Am J Kidney Dis 2009; 54: 159–164
7. Klockars M, Azar HA, Hermida R et al. The relationship of lysozyme to the nephropathy in chloroleukemic rats and the effects of lysozyme loading on normal rat kidneys. Cancer Res 1974; 34: 47–60
8. Santorielo D, Andal LM, Cox R et al. Lysozyme-induced nephropathy. Kidney Int Rep 2017; 2: 84–88
9. Pruzanski W, Platts ME. Serum and urinary proteins, lysozyme (muramidase), and renal dysfunction in mono- and myelomonocytic leukemia. J Clin Invest 1970; 49: 1694–1708
10. Selifverstova EV, Prutskova NP. Receptor-mediated endocytosis of lysozyme in renal proximal tubules of the frog Rana temporaria. Eur J Histochem 2015; 59: 79–86
11. Christensen EI, Verroust PJ, Nielsen R. Receptor-mediated endocytosis in renal proximal tubule. Pflugers Arch 2009; 458: 1039–1048
12. Hilderson I, Van Laecke S, Wauters A et al. Treatment of renal sarcoidosis: is there a guideline? Overview of the different treatment options. Nephrol Dial Transplant 2014; 29: 1841–1847
13. Near KA, Lefford MJ. Use of serum antibody and lysozyme levels for diagnosis of leprosy and tuberculosis. J Clin Microbiol 1992; 30: 1105–1110
14. Chapagain A, Dobbie H, Sheaff M et al. Presentation, diagnosis, and treatment outcome of tuberculosis-mediated tubulointerstitial nephritis. Kidney Int 2011; 79: 671–677

Received: 29.11.2019; Editorial decision: 15.3.2020