Tohoku J. Exp. Med., 2024, **264**, 53-60

Focal Light Chain Proximal Tubulopathy Complicated by Monoclonal Gammopathy of Undetermined Significance/ Smoldering Multiple Myeloma Successfully Diagnosed by Immunofluorescence on Pronase-Digested Paraffin Section: Reports of Two Cases and Review of the Literature

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Light chain proximal tubulopathy (LCPT) is a rare type of paraprotein-related disease (PRDs) characterized by monoclonal free light chain (FLC) deposition in proximal tubular epithelial cells (PTECs). A diagnosis of LCPT requires identification of FLC deposition in PTECs; however, FLC luminescence defects in immunofluorescence staining using frozen tissue (IF-F), regarded as "masked LCPT", are occasionally encountered. We describe two cases of focal masked LCPT in monoclonal gammopathy of undetermined significance (MGUS) or smoldering multiple myeloma (SMM) diagnosed by IF in formalin-fixed, paraffin-embedded tissue sections following pronase digestion (IF-P) rather than by IF-F. Case 1 was a 66-year-old woman who exhibited renal dysfunction with IgG-*λ* monoclonal proteinemia, and Case 2 was a 69-year-old man who exhibited renal dysfunction with IgG-*κ* type monoclonal proteinemia. In both cases, renal pathology showed focal tubular damage consisted of swelling and desquamation of PTECs. FLC deposition in PTECs was detectable by IF-P but not by IF-F. Consequently, an appropriate diagnosis by IF-P led the patients to receive chemotherapy immediately. These two cases indicate that LCPT can be present even if tubular injury is focal and PRD is not severe. According to a literature review of 33 cases, including our 2 cases, focal LCPT complicated by MGUS/SMM is relative rare. In PRD, evaluation with IF-P is desirable for assessing LCPT when FLC deposition is undetected by IF-F despite characteristic degenerative PTECs. We consider that early and definitive diagnosis of LCPT by IF-P rather than IF-F might result in favorite outcome since physicians could smoothly decide treatment strategy.

Keywords: focal tubular damage; light chain proximal tubulopathy; monoclonal gammopathy of undetermined significance; paraprotein-related disease; pronase digestion; smoldering multiple myeloma
Tohoku J. Exp. Med., 2024 September, **264** (1), 53-60.
doi: 10.1620/tjem.2024.J047

Introduction

Light chain proximal tubulopathy (LCPT) is a rare disease that presents with tubular injury due to deposition of free light chains (FLCs) in proximal tubules (PTs) (Kousios et al. 2023). Identification of FLC deposition in proximal tubular epithelial cells (PTECs) in renal biopsy (RB) specimens is essential for diagnosis. LCPT is mainly associated with paraprotein-related diseases (PRD) such as multiple myeloma (MM) and monoclonal gammopathy of undetermined significance (MGUS) (Doshi et al. 2016). LCPT was reported to occur in 0.5%-5.0% of patients with PRD who received RB (Gowda et al. 2015; Li et al. 2023). Clinical symptoms of LCPT include renal dysfunction, tubular proteinuria, and Fanconi syndrome. It was previously considered that the clinical course of LCPT progressed slowly;

Received April 11, 2024; revised and accepted June 11, 2024; J-STAGE Advance online publication June 20, 2024
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however, recent research has indicated that its progression can lead rapidly to end-stage renal failure (Stokes et al. 2016). A treatment strategy has not been established, but the choice and intensity depend on the severity of the causative PRD. Fundamentally, LCPT associated with MGUS might not be indicated for hematological treatment, in contrast to intensive treatment for MM-related LCPT. As described above, the renal prognosis in LCPT is not favorable. A previous study reported that therapeutic intervention in early-phase LCPT improved renal outcomes and recommended initiation of treatment before estimated glomerular filtration rate (eGFR) deteriorates, regardless of PRD severity (Stokes et al. 2016).

Histologically, diffuse swelling and desquamation of PTECs are commonly observed in LCPT. Damaged PTECs usually display degenerative changes characterized by loss of brush border, luminal ectasia, cytoplasmic simplification with vacuolization, and prominent nucleoli (Herlitz et al. 2009). Degenerative changes in PTECs tend to be accompanied by intracellular inclusions that appear pale with PAS staining and form multifocal cleft-like spaces (Herlitz et al. 2009; Stokes et al. 2016). Although FLC-produced crystals in tubules are a characteristic finding in LCPT, it is not rare to observe amorphous deposits or non-crystal formations in tubules (Kousios et al. 2023; Li et al. 2023). Therefore, detection of FLC deposits in PT with diffuse destructive tubular lesions is vital for a diagnosis of LCPT. The standard method for routine analysis of FLC deposition in LCPT is immunofluorescence staining using frozen tissue (IF-F). However, luminescence of FLCs is occasionally defective by IF-F even when FLCs are actually deposited in PTs. This false-negative IF staining for immunoglobulin light chains (LCs) in frozen sections is regarded as "masked LCPT."

Herein, we describe two cases of masked LCPT with focal tubular damage due to MGUS or smoldering MM (SMM) (Rajkumar et al. 2014) that were successfully diagnosed by IF in formalin-fixed, paraffin-embedded tissue sections following pronase digestion (IF-P), rather than by IF-F. As far as we know, masked LCPT with focal (not diffuse) tubular damage due to non-severe PRD, indicating MGUS or SMM, is deemed to be relative rare. We discuss the necessity of antigen activation by pronase-digested paraffin sections for detecting LCPT in PRD-related tubular damage. Additionally, 33 previously reported cases of LCPT complicated by MGUS and SMM (MGUS/SMM), as the present two cases, are reviewed, and the rarity and significance of the present two cases are highlighted.

Case Presentation

Patient 1

A 66-year-old woman with a history of breast cancer and HCV infection was admitted to our hospital because of aggravation of renal dysfunction. On admission, her temperature was 36.4°C, her pulse was 82 beats per minute, and her blood pressure was 148/72 mmHg. Findings on physical examination were unremarkable. Laboratory tests showed: white blood cell (WBC) count, 5,800/mm^3; erythrocyte count, 294 × 10^4/μL; hemoglobin, 9.1 g/dL; hematocrit, 28.2%; platelet count, 25.8 × 104/mm^3; total protein (TP), 8.3 g/dL; albumin (Alb), 4.4 g/dL; blood urea nitrogen (BUN), 29.7 mg/dL; creatinine (Cr), 1.4 mg/dL; eGFR, 30.0 mL/min/1.73 m^2; uric acid (UA), 10.8 mg/dL; total cholesterol, 180 mg/dL; sodium (Na), 142 mEq/L; potassium (K), 3.7 mEq/L; chloride (Cl); 104 mEq/L; calcium (Ca), 10.6 mg/dL; phosphorus (P), 4.2 mg/dL; C-reactive protein (CRP), 0.08 mg/dL; IgG, 2,195 mg/dL; IgA, 36 mg/dL; and IgM, 32 mg/dL. Serum complement levels were within normal limits. No anti-nuclear antibody and anti-neutrophil cytoplasmic antibodies were detected. Serum hepatitis B surface antigen and anti-hepatitis C virus RNA level were negative. Results of urinary analysis were as following; urine protein (UP), 1+ (15.8 g/g Cr); urinary N-acetyl-beta-glucosaminidase (NAG), 58.8 U/L; urinary β2-microgrobline (β2-MG) 56,700 μg/L. Urine occult blood and blood sugar were negative, and urinary Bence Jones protein (BJP) was positive. Urinary amino acid analysis showed no para-amino aciduria, and electrolytes were negative for Fanconi syndrome. Blood and urine protein fractionation analysis demonstrated M-peak in gamma globulin fraction, and IgG-λ M-protein was detected in immunoelectrophoresis-serum test. Free κ and λ chains were 22.5 mg/L and 13,900 mg/L, respectively. Bone marrow (BM) examination showed 6.2% plasma cells. Collectively, obtained laboratory data and BM findings were compatible with the diagnostic criteria of SMM (Rajkumar et al. 2014).

On day 2 after admission, percutaneous RB was performed to obtain a definitive diagnosis and evaluate the degree of PRD. Eighteen glomeruli were available, of which one showed global sclerosis. No glomeruli with crescent formation were seen. Light microscopic (LM) examination of glomeruli showed no obvious abnormalities in the mesangial area or glomerular basement membrane (GBM). LM of tubulointerstitium revealed focal tubular damage of enlarged proximal tubular epithelium in PTs (Fig. 1A-C). Focal round cell infiltration was also observed in tubulointerstitium, but no cast formation was apparent in PTs or distal tubules. Within the vasculature, only mild arterial intimal thickening was observed. Congo-red stain findings were negative. In analysis based on IF-F, no significant staining of IgG, IgA, IgM, C3, C4, C1q, κ, or λ (Fig. 1D) was found in glomeruli or tubular interstitium. In re-evaluation by IF-P, immunoglobulins, complement complements, and κ remained negative, but diffuse positive staining of λ was clearly seen in tubules (Fig. 1E, F). Destructive change of PTECs was confirmed in electron microscopy (EM) analysis (Fig. 2A). The cytoplasm of some tubular epithelial cells was swollen and loose, with a large number of swollen mitochondria and lysosomal granules, but no obvious crystal formation in the tubules (Fig. 2B). These findings indicated a diagnosis of LCPT of

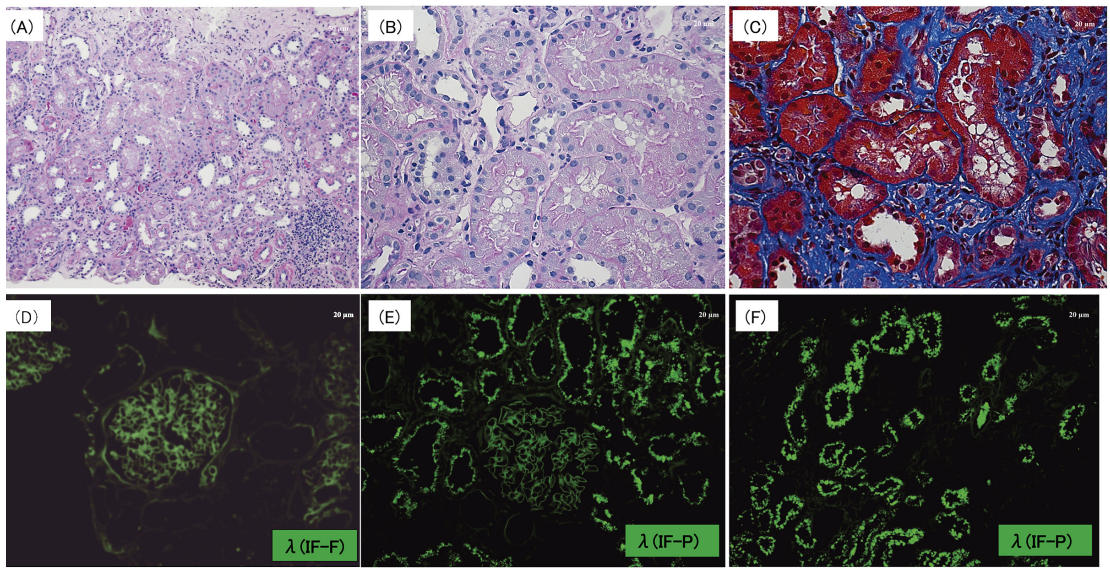

Fig. 1. Renal biopsy findings in Patient 1.
(A) Light microscopy shows focal tubulointerstitial damage (periodic acid-Schiff stain, × 40). (B) Light microscopy shows enlarged proximal tubular epithelium in proximal tubules (PTs) (periodic acid-Schiff stain, × 400). (C) PTs show vacuolar degeneration and partial dilatation (Masson trichrome stain, × 400). (D) Immunofluorescence staining using frozen tissue shows absence of λ expression in glomeruli and tubules. (E) IF on formalin-fixed, paraffin-embedded tissue sections following pronase digestion (IF-P) reveals diffuse positive staining of λ in tubules. (F) Significant positive staining of λ by IF-P is remarkable in tubules.

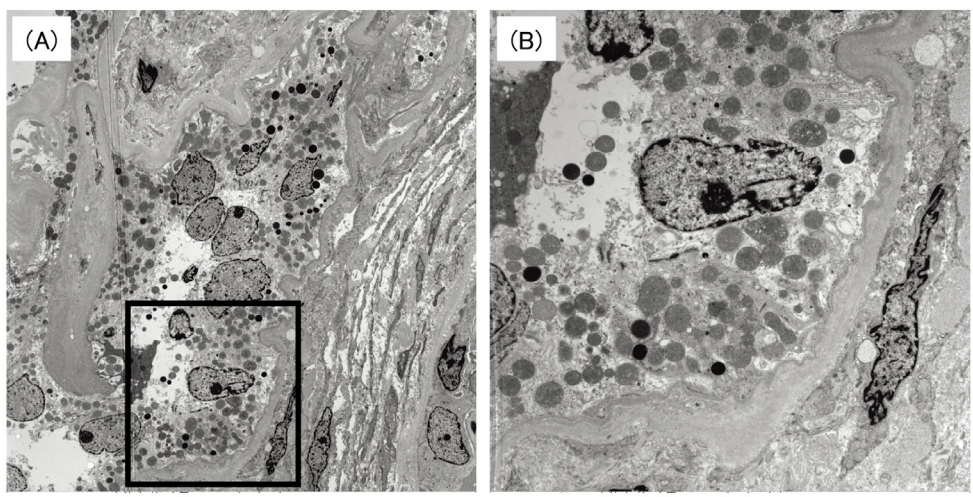

Fig. 2. Electron microscopic findings in Patient 1.
(A) Destructive change of proximal tubular epithelium is apparent. The cytoplasm of some tubular epithelial cells is swollen with numerous mitochondria and lysosomal granules (original magnification × 2,500). (B) An enlarged view of tubules in the square area of (A) shows no obvious crystal formation in tubules (original magnification × 7,000).

FLC-κ without crystal formation.

Thereafter, on day 77 after admission, the patient received VD therapy consisted of bortezomib 1.3 mg/m² and dexamethasone 20 mg/body against biopsy-proven LCPT associated with SMM, which reduced the level for FLC-λ. However, the elevation of FLC-λ level was flared up on day 120 after admission, regimens for chemotherapy was change to VRd therapy (bortezomib 1.3 mg/m², lenalidomide 10 mg/body, and dexamethasone 20 mg/body). Subsequently, despite the change of regimens for chemotherapy, the level for FLC-λ remained still elevated, thereby we changed the regimen for chemotherapy again from VRd to Dkd therapy (daratumumab 16 mg/kg, carfilzomib 56 mg/ m² , dexamethasone 20 mg/body) on day 170 after admission. Consequently, the patient achieved clinical remission in which FLC-λ level was disappeared and urinary tubular protein composed of M-protein was markedly decreased (Fig. 3). Additionally, the patient's renal function was not deteriorated by the chemotherapy during her clinical course.

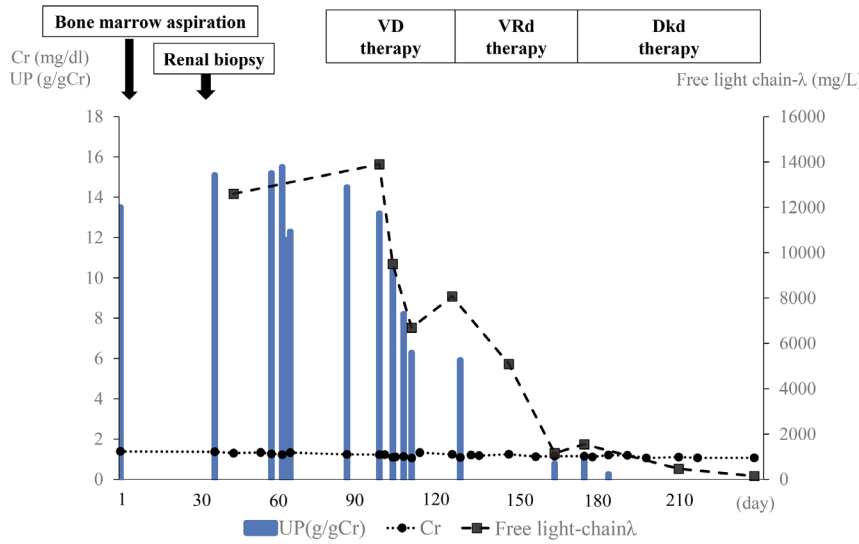

Fig. 3. Clinical course in Patient 1.
UP, urinary protein; Cr, creatinine: VD, bortezomib dexamethasone; VRd, bortezomib lenalidomide dexamethasone; DKd, daratumumab carfilzomib dexamethasone.

Patient 2

A 69-year-old man who already had been diagnosed as MGUS in another hospital was admitted to our hospital in order to examine aggravated renal disorder. On admission, his temperature was 36.5°C, his pulse was 72 beats per minute, and his blood pressure was 128/77 mmHg. Findings on physical examination were unremarkable. Laboratory tests showed: WBC, 3,600/mm^3; erythrocyte count, 527 × 10^4/μL; hemoglobin, 15.6 g/dL; hematocrit,48.3%; platelet count, 19.3 × 104/mm^3; TP, 7.1 g/dL; Alb, 4.0 g/dL; BUN, 22.3 mg/dL; Cr, 1.4 mg/dL; eGFR, 43.0 mL/min/1.73 m^2; UA, 1.8 mg/dL; total cholesterol, 251 mg/dL; Na, 137 mEq/L; K, 3.8 mEq/L; Cl, 103 mEq/L; Ca, 8.8 mg/dL; P, 3.1 mg/dL; CRP, 0.03 mg/ dL; IgG, 1,681 mg/dL; IgA, 107 mg/dL; and IgM, 43 mg/dL. Serum complement levels were within normal limits. No antinuclear antibody and anti-neutrophil cytoplasmic antibodies were detected. Urinary findings were as following; UP, 1+ (0.51 g/g Cr), urine sugar, 1+; urine occult blood, negative; urinary NAG, 12.8 U/L; urinary β2-MG 414 μg/L. Urinary amino acid analysis revealed para-amino aciduria indicative of Fanconi syndrome. M-peak in blood and urine protein fractions was apparent, and IgG-κ M-protein was detected in immunoelectrophoresis-serum test. Free κ and λ chains were 115 mg/L and 13.9 mg/L, respectively. Additionally, κ/λ ratio was 8.29 and indicated elevation of monoclonal FLC κ. Moreover, BM examination showed 1.4% plasma cells, indicating MGUS.

On day 2 after admission, we performed RB to assess the degree of PRD and evaluate the presence of LCPT. Forty glomeruli were available, of which one showed global sclerosis. No crescent formation was seen. LM revealed no obvious abnormalities in the mesangial area or GBM. Tubulointerstitium showed focal tubular damage in PTs with enlarged proximal tubular epithelium and loss of brush border (Fig. 4A-C). The degenerative change in PTECs was accompanied by intracellular pale PAS staining (Fig. 4B). Masson staining positive red depositions were also detected in some parts of PTECs with degenerative change (Fig. 4C). Focal cellular infiltration was detected but no apparent crystal formation was detected in tubules. No remarkable features of arteritis were noted. In IF-F analysis, immune-related substances including IgG, IgA, IgM, C3, C4, C1q, κ (Fig. 4D), and λ were all negative in glomeruli and tubulointerstitium. Repeat analysis with IF-P revealed diffuse κ deposition in tubules (Fig. 4E, F). EM demonstrated damaged PTECs with deposition of rhombic and trapezoidal crystals (Fig. 5A-C). The cytoplasm of damaged tubular epithelial cells contained numerous swollen mitochondria and lysosomal granules fused into irregular mottled shapes. In addition, PTECs contained intracytoplasmic crystalline inclusions (Fig. 5B) and the intracytoplasmic inclusions appeared to have a fibrillar component. (Fig. 5C). EM showed no evidence of luminal or fibril structures in glomeruli. Collectively, the findings indicated a diagnosis of LCPT due to monoclonal FLC-κ deposition with crystal formation. We informed the patient of such definitive diagnosis of LCPT and proposed the priority of intensive therapy against MGUS that is a cause of LCPT. Eventually, on day 70 after admission, the patient is scheduled to be transferred to the hematology department of the referral hospital and then receive chemotherapy against MGUS.

Consent for publication

Informed consents were obtained from the patients described in present two cases.

Discussion

In both patients, renal disorder with focal tubular dam-

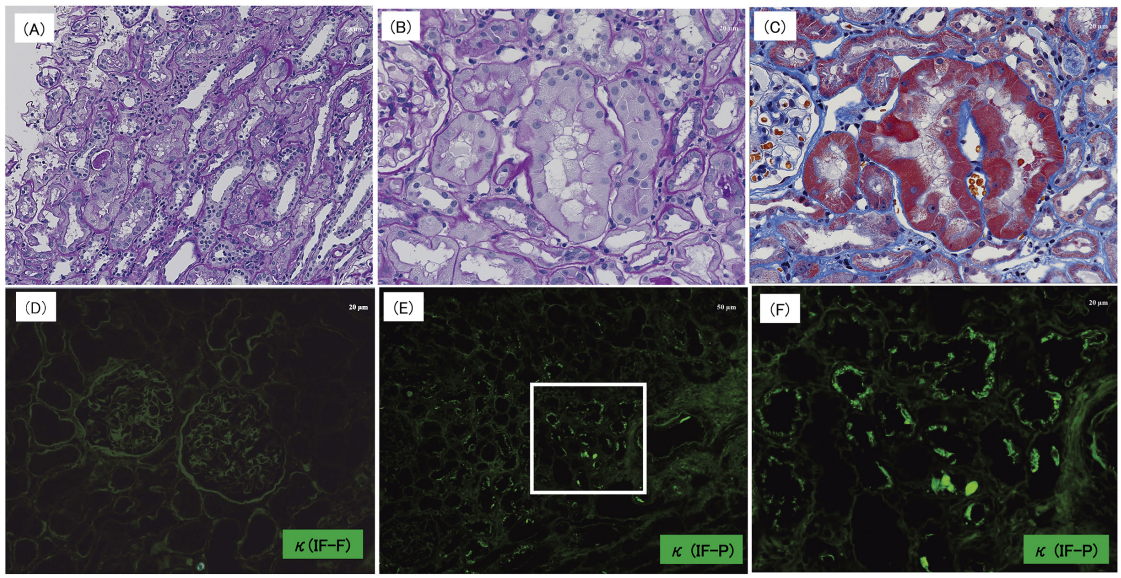

Fig. 4. Renal biopsy findings in Patient 2.
(A) Light microscopy shows focal tubulointerstitial damage (periodic acid-Schiff stain, × 100). (B) Light microscopy shows tubular damage in proximal tubules (PTs) with enlarged proximal tubular epithelium and loss of brush border. The degenerative change in PTs is accompanied by intracellular pale staining (periodic acid-Schiff stain, × 400). (C) Masson staining positive red depositions are detected in some parts of PTs with degenerative change (Masson trichrome stain, × 400). (D) Immunofluorescence staining using frozen tissue shows absence of κ expression in tubules. (E) IF on formalin-fixed, paraffin-embedded tissue sections following pronase digestion (IF-P) reveals focal and scattered positive expressions of κ in tubules. (F) In an enlarged view of tubules in the square area of (E), IF-P clearly shows staining of κ in PTs.

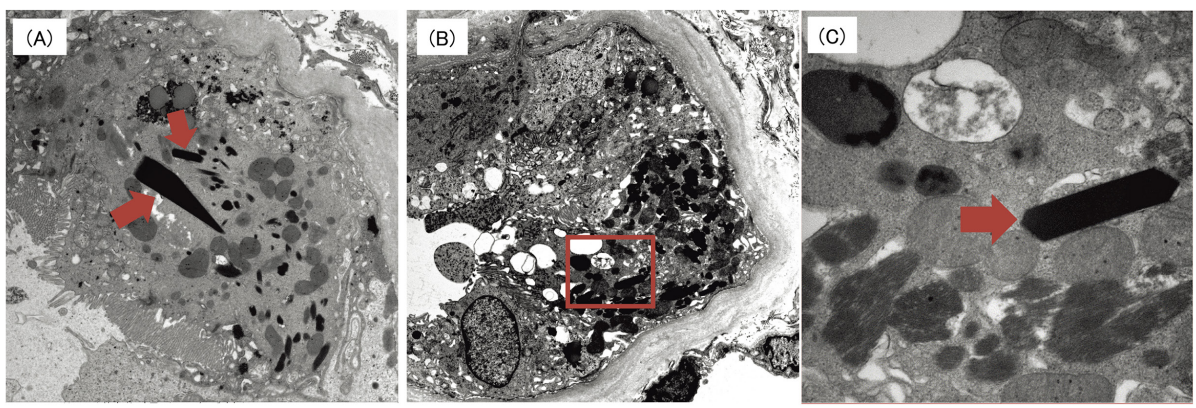

Fig. 5. Electron microscopic findings in Patient 2.
(A) Damaged proximal tubular cells contain deposition (arrows) of rhombic and trapezoidal crystals (original magnification × 8,000). (B) Cytoplasm of tubular epithelial cells is swollen with many swollen mitochondria and lysosomal granules fused into irregular mottled shapes. Proximal tubular cells contain intracytoplasmic crystalline inclusions (original magnification × 6,000). (C) An enlarged view of tubular epithelial cells in the square area of (B) demonstrates intracytoplasmic inclusions which is appeared to have a fibrillar component (arrow).

age was considered attributable to LCPT, even though the complicating PRD was MGUS or SMM rather than MM, and the extent of tubular damage was focal rather than diffuse. To the best of our knowledge, MGUS/SMM related LCPT with focal tubular damage is relative rare. According to a literature review of 33 cases including the present two cases (Table 1), 3 (11.1%) of 27 cases, in which extent of tubular injury was evaluated, showed focal MGUS-associated LCPT (Stokes et al. 2016; Ito et al. 2019; Patel et al. 2020; Sugimoto et al. 2021; Lindemann et al. 2021; Shao et al. 2021; Terinte-Balcan et al. 2022; Li et al. 2023; Kono et al. 2023; Tang et al. 2023; Tsuyuki et al. 2024) . Therefore, it is critical to keep in mind the possibility that the tubular damage in patients with PRD might be attributable to LCPT even though the extent of tubular injury is focal on pathological findings.

In clinical practice, renal disorder is commonly not severe unless the patient's complicating PRD is MM. Renal

Table 1. Clinical features of previously reported cases of light chain proximal tubulopathy due to monoclonal gammopathy of undetermined significance or smoldering multiple myeloma.

No.	Ref	Age (y)/ sex	LC isotype	Chemotherapy regimen	Pre/post chemotherapy FLC (mg/dL)	Pre/post chemotherapy sCr (mg/dL)	Pre/post chemotherapy UP (g/day)	Detection by IF-F	Detection by IF-P	Extent of tubular injury	Crystals	Renal outcome
1	Stokes et al. (2016)	76/NA	κ	NA	NA	3.3/NA	2.0/NA	-	+	d	+	NA
2	Stokes et al. (2016)	43/NA	κ	NA	NA	1.8/NA	7.0/NA	-	+	d	unknown	NA
3	Stokes et al. (2016)	68/NA	κ	none	NA	1.9/10	6.7/NA	+	+	d	+	ESRD
4	Stokes et al. (2016)	58/NA	κ	NA	NA	1.5/NA	3.5/NA	-	+	d	+	NA
5	Stokes et al. (2016)	49/NA	κ	NA	NA	1.4/NA	7.25/NA	NA	+	d	+	NA
6	Stokes et al. (2016)	81/NA	κ	none	NA	4.4/NA	0.75/NA	-	+	d	+	ESRD
7	Stokes et al. (2016)	75/NA	κ	NA	47.6/NA	2.5/NA	2.9/NA	-	+	d	+	NA
8	Stokes et al. (2016)	72/NA	κ	B,D	NA/23	1.5/1.6	4.0/NA	not done	+	d	+	CKD
9	Stokes et al. (2016)	85/NA	κ	D	NA	6.0/2.0	4.0/1.5	-	+	d	+	CKD
10	Stokes et al. (2016)	52/NA	κ	M	NA	4.4/2.84	1.0/0.3	+	+	d	+	CKD
11	Stokes et al. (2016)	47/NA	κ	B,D	8.2/15.8	2.2/3.1	2.6/2.6	-	+	d	+	CKD
12	Stokes et al. (2016)	65/NA	κ	B	NA	2.7/5.0	2.0/NA	-	+	d	+	CKD
13	Stokes et al. (2016)	51/NA	κ	B, D, L, M	NA/1.16	1.5/1.55	2.3/0.8	-	+	f	+	CKD
14	Stokes et al. (2016)	44/NA	κ	NA	NA	3.3/NA	2.39/NA	-	+	d	+	NA
15	Stokes et al. (2016)	81/NA	κ	NA	NA	2.7/NA	5.5/NA	-	+	d	+	NA
16	Stokes et al. (2016)	81/NA	κ	T,B	18.5/2.3	2.5/3.42	2.9/0.9	+	+	d	+	CKD
17	Stokes et al. (2016)	53/NA	κ	NA	NA	2.0/NA	0.9/NA	-	+	d	+	NA
18	Stokes et al. (2016)	71/NA	κ	B, D, C	33.7/47.6	1.5/2.39	3.3/1.82	-	+	d	+	CKD
19	Stokes et al. (2016)	74/NA	κ	NA	NA	2.9/NA	1.76/NA	-	+	d	+	NA
20	Stokes et al. (2016)	58/NA	κ	none	NA	1.3/NA	1.28/NA	-	+	d	+	NA
21	Stokes et al. (2016)	87/NA	κ	T	NA/4.5	4.0/1.09	0/0.08	-	+	d	+	NA
22	Ito et al. (2019)	65/F	κ	B, D	NA	1.94/NA	1.31/NA	NA	NA	d	+	improved
23	Patel et al. (2020)	64/M	κ	B, D, C	4.87/NA	1.69/NA	2.94/NA	NA	+	NA	+	ESRD
24	Sugimoto et al. (2021)	82/M	κ	none	NA	0.83/NA	0.74/NA	NA	NA	NA	+	CKD
25	Shao et al. (2021)	72/M	κ	NA	4140/NA	8.02/NA	2.1/NA	-	+	NA	-	NA
26	Lindemann et al. (2021)	49/M	κ	B, D	60.6/NA	0.83/NA	1.20/NA	NA	NA	NA	+	CKD
27	Terinte-Balcan et al. (2022)	60/F	κ	B, D, C	152.8/	1.7/NA	5.4/NA	NA	NA	NA	+	NA
28	Kono et al. (2023)	64/M	κ	B, D, C	567/24.9	2.91/1.87	1.33/NA	NA	NA	NA	+	improved
29	Li et al. (2023)	67/M	κ	B, D	4.83/4.03	1.33/1.31-1.42	1.18/NA	NA	NA	d	-	CKD
30	Tang et al. (2023)	49/F	κ	B, D, C	485/NA	4.4/NA	5.44/NA	not done	+	d	-	improved
31	Tsuyuki et al. (2024)	70/F	λ	B, D	162.5/29.2	3.91/1.35	1.59/0.82	-	+	NA	-	improved
32	The present case 1	66/F	λ	B, D, L, DA, CA	13900/145	1.4/1.07	15.8/0.27	-	+	f	-	CKD
33	The present case 2	69/M	κ	NA	115/NA	1.4/NA	0.51/NA	-	+	f	+	NA

Ref: reference, y: years, M: male, F: female, LC: light chain, B: Bortezomib, D: dexamethasone, M: melpharan, L: lenalidomide, T: Thalidomide, C: cyclophosphamid, DA: daratumumab, CA: carfilzomib, FLC: free light chain, sCr: serum creatinin, UP: urinary protein, IF-F: immunofluorescence staining using frozen tissue, IF-P: immunofluorescence staining using pronase-digested paraffin section, d: diffuse, f: focal, ESRD: end stage renal disease, CKD: chronic kidney disease, NA: not available.

damage in MGUS tends to manifest as tubular proteinuria only, which may discourage physicians from performing RB. This non-active attitude to RB in MGUS may be the cause of the low prevalence rate of LCPT in PRD. Therefore, early and proactive RB to evaluate the presence of LCPT might be critical, even if renal disorder in PRD is not high priority when considering the clinical course and renal outcome in LCPT. Moreover, LCPT due to incomplete evaluation by IF-F may be also related to the low prevalence of LCPT. Indeed, as shown in Table 1 which reviews the previously reported LCPT complicated by MGUS/SMM, 20 (86.9%) of 23 cases evaluated by both IF-F and IF-P analysis were masked LCPT, and the proportion of cases appropriately diagnosed as LCPT by IF-F was low (13.0%, 3/23 cases). Thus, it is critical to prevent false-negative findings of LCPT in IF analysis, even in the case of focal tubular damage, as in the two present cases.

Of note, IF-P analysis provided a clear diagnosis of LCPT in both patients, enabling early decision of chemotherapy. We considered that immediate decision to perform chemotherapy against MGUS/SMM associated LCPT based on an appropriate diagnosis by IF-P might lead to favorite outcome in Case1 patient. Regarding the treatment strategy for LCPT complicated by MGUS/SMM, strong evidence has not been established. However, in our review of literature (Table 1), renal outcome tends not to be poor in the cases receiving the intensive chemotherapy. In fact, aggressive chemotherapy in the present Case 1 resulted in achievement of hematological partial remission without aggravating renal dysfunction, which may indicate the necessity of considering aggressive therapy against LCPT complicated by MGUS/SMM. However, the efficacy and safety of intensive chemotherapy against MGUS/SMM remains still elusive. Persistent immunosuppression status after early initiation of aggressive chemotherapy might be a high risk. Thus, considering appropriate treatment strategy based on the patient's clinical background is indispensable.

FLCs filtrated from glomeruli are usually taken up endosomally via megalin/cubilin scavenger receptors in PTECs and then degraded by lysosomes (Sanders 2012). However, overproduced FLCs that exceed the absorption capacity induce tubular injury via activation of inflammatory cytokines, ultimately causing crystalline formation (Sanders 2012; Stokes et al. 2016). LCPT is classified as crystalline and non-crystalline based on the presence or absence of crystal structures in the tubular cytoplasm. In a previous study of 46 patients with LCPT, 87% were crystalline and 13% were non-crystalline (Stokes et al. 2016). Similarly, as shown in our summarizing Table 1, 84% were crystalline and 16% were non-crystalline in the 33 cases of LCPT complicated by MGUS/SMM. Histologically, there is no crucial difference between the two forms under LM, but FLC-crystallinity was reported to be higher in the case of kappa-chain deposition, as similarly shown in our review of literature (Table 1), because the Vκ domain of monoclonal propagated kappa-chains is resistant to cathepsin B and proteolytic enzymes in lysosomes (Leboulleux et al. 1995). Under EM, accumulated LCs in crystalline LCPT appear as rhomboid or needle-like crystals, whereas excess LC deposition in non-crystalline LCPT is apparent as mottled electron-dense particles in dysmorphic lysosomes (Li et al. 2023). Crucially, several studies have demonstrated that excess FLCs can induce tubular injury even without crystal formation (Sanders et al. 1987; Sanders et al. 1988; Sengul et al. 2002). Thus, a definitive diagnosis is necessary for LCPT regardless of the presence of crystalline structures, and reliable IF analysis is key for diagnosis because determination of non-crystalline LCPT using methods other than IF analysis can be difficult for non-renal pathologists.

A previous study reported that in most cases of crystalline LCPT, IF-P was required to detect monoclonal LCs, whereas almost cases of non-crystal LCPT could be detected by IF-F (Nasr et al. 2006). In the present cases, non-crystalline FLC deposition was not detected in patient 1 by IF-F, and both cases required IF-P for diagnosis. Although the reason for the discrepancy between the previous and the present cases is unclear, IF-P might be superior to standard IF-F for determining the LC composition of PTs, with or without crystals. Several studies have emphasized the value of IF-P as a diagnostic tool for LCPT, particularly as un unmasking tool (Messias et al. 2015; Stokes et al. 2016; Nasr et al. 2018). Stokes et al. (2016) reported a detection sensitivity of LCPT by IF-P of 97% (37/38 cases) versus 35% (15/43 cases) by IF-F. In short, IF-P increases detection sensitivity and can therefore provide a reliable definitive diagnosis when LCPT is masked by IF-F. In contrast to cryostat sections cut from frozen tissue, paraffin-embedded sections fixed in formalin are suitable for preservation of tissue morphology (Nasr et al. 2018). It has been postulated that an appropriate antigen-retrieval step using trypsin, protease VII, protease XXIV, or pronase increases penetration of antibodies to the antigens (Fogazzi et al. 1989; Nasr et al. 2018). Pronase digestion has a particularly powerful denaturing effect on cell membranes (Nasr et al. 2018) that can uncover sequestered antigenic sites. However, it is not necessary to perform IF-P in all cases. Indeed, IF-F remains the gold standard immunohistochemical technique for RB. If there is discordance between the LM, EM, and IF-F findings in investigating LCPT, masked deposits should be considered and evaluation should be performed with IF-P rather than IF-F.

In conclusion, it is critical that IF analysis is performed to evaluate the presence of LCPT, even if PRD is neither severe nor extended. IF-P analysis is desirable to obtain a definitive diagnosis in cases when FLC deposition is undetermined by routine IF-F despite characteristic proximal tubular changes. Moreover, we suggest that early and definitive diagnosis of LCPT by IF-P rather than IF-F could lead physicians to decide treatment strategy smoothly, which might result in favorite outcome of LCPT.

Acknowledgements

We would like to thank to Ms. Naoko Ishigaki for her excellent technical assistance.

Conflict of Interest

The authors declared no conflict of interest.

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