Posttransplant nephrotic syndrome resulting from NELL1-positive membranous nephropathy

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Membranous nephropathy (MN) constitutes a major cause of nephrotic syndrome (NS) in adults. After kidney transplantation (KTx), both recurrent and de novo MN has been reported. In addition to PLA2R and THSD7A, recent identification of neural EGFL-like-1 protein, NELL1, as a potential disease antigen has enriched our understanding of MN pathogenesis. To date, NELL1-positive MN has only been described in native kidneys, but never been diagnosed in renal allografts. We here report on a 56-year-old male kidney transplant recipient suffering from amyotrophic lateral sclerosis (ALS), who developed NS 25 years after KTx. Allograft biopsy revealed NELL1-positive MN. Using specifically established immunoblotting techniques, we detected new-onset NELL1-IgG1, IgG3, and IgG4 antibodies in the patient’s serum correlating with the course of proteinuria. While primary renal disease was undetermined, MN recurrence seemed unlikely given the long-time span since KTx. By clinical investigation of de novo etiologies, we did not detect an underlying malignancy. However, previous self-medication with dimercaptopropane sulfonate (DMPS) and alpha lipoic acid (ALA) represented a potential trigger and cessation associated with partial remission of proteinuria. This report illustrates the first case of posttransplant NS due to NELL1-positive MN. Monitoring NELL1 antibodies in the serum promise to be a non-invasive diagnostic tool guiding disease management.

KEYWORDS
autoantibody, autoantigen, basic (laboratory) research/science, clinical research/practice, glomerular biology and disease, kidney (allograft) function/dysfunction, kidney disease: immune/inflammatory, kidney transplantation/nephrology, molecular biology, pathology/histopathology

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1 | INTRODUCTION

Membranous nephropathy (MN) is an immune-mediated glomerular disease constituting a major cause of nephrotic syndrome (NS) in adults. One fifth of cases is attributed to secondary causes, such as infections, malignancies, or drugs, whereas the majority is considered as primary disease with no further disease association. Formation of subepithelial immune deposits, containing antibody-antigen complexes and complements components, is the characteristic histological finding in renal biopsies. Despite the landmark identification of phospholipase A2 receptor 1 (PLA2R) and thrombospondin type 1 domain-containing protein 7a (THSD7A) as major target antigens, about 40% of both primary and secondary MN cases remained unexplained. By the use of laser microdissection and tandem mass spectrometry, further potential MN antigens, such as exostosin 1/exostosin 2 (EXT1/EXT2), semaphorin 3b (Sema3B), and neural EGFL-like-1 protein (NELL1), were recently discovered. The detection of PLA2R and THSD7A as MN antigens in renal specimen and the respective autoantibodies in the blood enabled improvements in disease surveillance and allowed for stratified diagnostic and treatment strategies, for example, screening for malignancies in THSD7A-positive or PLA2R-negative MN. Despite diagnostic and therapeutic advancements, about 20% of affected patients progress to end-stage kidney disease (ESKD) requiring renal replacement therapies. A limitation of kidney transplantation (KTx) in MN is the risk of disease recurrence in renal allografts, ranging from 20% to 50% of cases.

Posttransplant MN can occur as de novo or recurrent disease, and anecdotal cases of posttransplant MN have been published as donor-derived disease. Antibody-mediated rejection and viral infections are considered as additional secondary causes of posttransplant MN. Recent studies indicate that in PLA2R-positive MN, the occurrence of specific antibodies is associated with an increased risk for allograft MN. In contrast, reliable data regarding the post-transplant recurrence of other primary MN entities, such as NELL1-positive MN, are unavailable.

2 | CASE REPORT

We here report on a 56-year-old male kidney transplant patient, who was admitted due to new-onset NS 25 years after successful KTx (serum creatinine 152 µmol/L, eGFR$_{CKD-EPI}$ 54 mL/min/1.73 m$^2$, proteinuria 4041 mg/g creatinine, and serum albumin 23.8 g/L) (Figure 1A). His steroid-free immunosuppressive regimen consisted of 1.5 mg tacrolimus and 1000 mg mycophenolate mofetil per day. KTx function had previously been stable and uncomplicated over decades.

Twelve months prior to admission, however, the patient was diagnosed with amyotrophic lateral sclerosis (ALS) with bulbar onset. ALS diagnosis was based on clinical presentation and typical electromyogram and neurogram recordings. Biochemical blood and cerebrospinal fluid analysis, as well as cerebral magnetic resonance imaging yielded unremarkable results. Due to suspected ALS, a therapy with riluzole was initiated. At that time, allograft function was unaffected (serum creatinine 64 µmol/L, eGFR$_{CKD-EPI}$ 112 mL/min/1.73 m$^2$, and proteinuria <80 mg/g creatinine). Six months later, the patient refused further riluzole therapy and initiated an unapproved therapeutic attempt on his own authority. Hereby, a daily chelation therapy with alpha lipoic acid (ALA) and dimercaptopropane sulfonate (DMPS) was commenced 8 weeks prior to first NS diagnosis.

To uncover the underlying cause of new-onset NS, we performed a kidney transplant biopsy. Histological examination revealed both KM55 (antibody against galactose-deficient IgA1)-positive IgA nephropathy and subepithelial immune deposits, characteristic for MN (Figure 2A-G). While immunostaining for PLA2R and THSD7A remained negative, we obtained strong glomerular positivity for NELL1 (Figure 2H). Electron microscopy yielded foot process effacement and subepithelial localization of electron-dense deposits (Figure 2G) confirming the histological diagnosis of posttransplant NELL1-positive MN.

Due to the lack of commercially available assays, we newly established NELL1-immunoblotting under non-reducing conditions to first analyze whether NELL1 antibodies were present in the serum of the patient at the time of MN diagnosis (detailed methods are available in Supplementary File S1). Thereby, we found positivity for NELL1 total IgG antibodies (Figure 1B,C) as well as for the IgG subclasses IgG1, IgG3, and IgG4 (Figure 1D). Interestingly, NELL1 antibodies have been reported to most commonly belong to the IgG1 subclass, in difference to PLA2R and THSD7A antibodies, which are typically IgG4 subclass antibodies. However, in this case, we were able to identify NELL1-specific IgG4 antibodies in the serum, and IgG4 staining of the kidney biopsy was positive (Figure 2F). In contrast, neither PLA2R nor THSD7A antibodies were detectable in the patient’s serum. We were also able to analyze a serum sample, which was collected from the patient 31 months prior to the MN diagnosis. Remarkably, no NELL1 antibodies were detectable in the serum of the patient at that time (Figure 1C).

Clinical investigation for secondary causes including chest radiography, abdominal and whole-body lymph node sonography, gastroscopy as well as tumor marker analysis (e.g., prostate-specific antigen, CA19-9, and CEA) yielded no evidence of solid or lymphoproliferative malignancies. Screening for viral infections (including hepatitis B/C) was unremarkable, despite the detection of low plasma concentrations of Epstein-Barr virus. However, fluorescence-activated cell staining (FACS) analysis did not indicate lymphoproliferative disease. Lastly, human leukocyte antigen (HLA) antibodies remained absent, excluding significant antibody-mediated rejection in line with renal histopathology.

As neither native kidney biopsy material nor medical records regarding the patient’s primary renal disease were available, and given the long time range from KTx to onset of NS (25 years), we classified MN as de novo disease. Therapeutically, we initiated RAAS blockade (captopril followed by irbesartan) together with further supportive measures (torasemide and atorvastatin) and stopped DMPS and ALA.
intake while leaving the immunosuppressive regimen unchanged. After a follow-up time of 4 months, partial remission of proteinuria was noted, characterized by decreased proteinuria (2062 mg/g creatinine), normal serum albumin levels (39.4 g/L), and normal serum creatinine (71 µmol/L, eGFR C K D - E P I 97 mL/min/1.73 m²) (Figure 1A). Partial remission of proteinuria was accompanied by disappearance of circulating NELL1 antibody in the serum of the patient (Figure 1C).

3 | DISCUSSION

NELL1 (neural EGFL-like-1) is a 90 kDa secreted protein with highest expression in neural tissues and osteoblasts, and to a lower proportion in renal tubular cells, but barely in glomeruli under normal circumstances (uniprot.org/uniprot/Q92832 / gtexportal.org/home/gene/NELL1 / proteinatlas.org/ENSG00000165973-NELL1/ tissue). NELL1 consists of a secretory signal peptide followed by an amino-terminal laminin G-like domain, a coiled-coil domain, and six EGF-like repeats, which are flanked by von Willebrand domains. In contrast to PLA2R and THSD7A, NELL1 does not contain a transmembrane domain but is secreted. To date, estimated incidence of NELL1-positive MN varies between 4% and 16% of PLA2R-negative MN cases. Unlike in native kidneys, NELL1-positive MN has never been reported in KTx recipients. Post-KTx, MN is the second most common cause of nephrotic range proteinuria after transplant glomerulopathy. A majority of MN in renal allografts is due to recurrence of primary renal disease, estimated to account for 9%–45% of cases.

As has been shown in PLA2R-associated MN, where PLA2R antibody levels may be helpful in predicting disease recurrence, we demonstrate the presence of NELL1 antibodies in the patient’s serum (Figure 1C) at the time of MN diagnosis. In this patient, unfortunately, the primary kidney disease was unknown and frozen serum prior to KTx or previously obtained native kidney biopsy

![Figure 1](image_url)
material was unavailable in order to confirm or exclude disease recurrence. However, given the large time span between KTx and NS onset (25 years), we suspect de novo disease. Interestingly, analyses of NELL1 antibodies in serum probes collected 31 months prior to and 4 months after first MN diagnosis yielded negative results (Figure 1C). In conclusion, the presence of NELL1 antibodies in the serum correlated with the clinical findings of urinary protein excretion and might be of predictive value preceding complete remission (Figure 1A,C).

Upon glomerular NELL1-positivity in biopsy specimens, the question of primary versus secondary MN remains. To date, the majority of NELL1-positive cases in native kidneys is considered as primary MN. However, concurrent malignancies were found in as many as 33% of MN patients with glomerular NELL1 expression in native kidney biopsies. Whether these findings represent disease associations or mere coincidences is not yet established. Our examinations did not reveal any signs of an underlying malignancy at the time of first NS onset and up to 4 months follow-up.

Surprisingly, we found an IgA nephropathy in addition to MN in our patient. The scenario of coexisting IgA nephropathy and MN has been described previously in native kidneys and it is speculated that this coexistence represents two separate glomerular diseases rather than an atypical subclass of IgA nephropathy or MN. In line with this hypothesis, we found IgA (and KM55) deposition only in the mesangial compartment, whereas granular IgG- and NELL1-positivity were restricted to glomerular basement membranes. While KM55 positivity in renal specimen is associated with galactose-deficient IgA1, it is controversial whether this immunohistological pattern is able to distinguish primary from secondary IgA nephropathy.

Interestingly, the diagnosis of sporadic ALS, a fatal neurodegenerative disease of the first motor neuron, preceded the onset of NS by several months only. NELL1 is predominantly expressed in cells of the nervous system and recent data from a large genome-wide association study identified NELL1 among 51 risk loci for sporadic ALS. Nevertheless, a causal link between ALS and NELL1-positive MN remains purely speculative, as there was no evidence for an inflammatory or antibody-driven neurological disease and previous associations between MN and ALS are not reported in the literature.

Furthermore, it is tempting to speculate about disease-causing effects of administered agents. An impact of DMPS and/or ALA administration on MN onset in terms of an immunological trigger cannot be excluded and appears conceivable for its coincidence. Interestingly, an association of chelation agents, such as penicillamine, with MN has been previously reported though not been described for DMPS or ALA in particular.

To the best of our knowledge, we here report on the first case of NELL1-positive MN in a KTx recipient. In the absence of cancer and unlikely recurrence, we assumed primary de novo disease. Both clinical course and associated NELL1 antibody levels suggest their diagnostic and predictive value, similar to previously established PLA2R antibody titers. Consequently, monitoring NELL1 antibody levels in the serum may represent a novel non-invasive tool in differential diagnosis and therapeutic guidance in patients with NS.

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DISCLOSURE

The authors of this manuscript have no conflicts of interest to disclose as described by the American Journal of Transplantation.

DATA AVAILABILITY STATEMENT

The authors confirm that the data supporting the findings of this case report are available upon request.

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REFERENCES

1. Leon J, Pérez-Sáez MJ, Batal I, et al. Membranous nephropathy posttransplantation: an update of the pathophysiology and management. Transplant. 2019;103(10):1990-2002. https://doi.org/10.1097/TP.0000000000002758.
2. Beck LH, Bonegio RGB, Lambeau G, et al. M-type phospholipase A2 receptor as target antigen in idiopathic membranous nephropathy. N Engl J Med. 2009;361(11):2277-2287. https://doi.org/10.1056/NEJMoal0810457.
3. Tomas NM, Beck LH, Meyer-Schweisinger C, et al. Thrombospondin type-1 domain-containing 7A in idiopathic membranous nephropathy. N Engl J Med. 2014;371(24):2277-2287. https://doi.org/10.1056/NEJMoa1409354.
4. Sethi S, Madden BJ, Debiec H, et al. Exostosin 1/exostosin 2-associated membranous nephropathy. J Am Soc Nephrol. 2019;30(6):1123-1136. https://doi.org/10.1681 ASN.2018080852.
5. Sethi S, Debiec H, Madden B, et al. Semaphorin 3B-associated membranous nephropathy is a distinct type of disease predominantly present in pediatric patients. Kidney Int. 2020;98(5):1253-1264. https://doi.org/10.1016/j.kint.2020.05.030.
6. Sethi S, Debiec H, Madden B, et al. Neural epidermal growth factor-like 1 protein (NELL-1) associated membranous nephropathy. Kidney Int. 2020;97(1):163-174. https://doi.org/10.1016/j.kint.2019.09.014.
7. Hoxha E, Beck LH, Wiech T, et al. An indirect immunofluorescence method facilitates detection of thrombospondin type 1 domain-containing 7A-specific antibodies in membranous nephropathy. J Am Soc Nephrol. 2017;28(2):520-531. https://doi.org/10.1681 ASN.2016010050.
8. Filippone EJ, Farber JL. Membranous nephropathy in the kidney allograft. Clin Transplant. 2016;30(11):1394-1402. https://doi.org/10.1111/clt.12847.
9. Mirza MK, Kim L, Kadambi PV, Chang A, Meehan SM. Membranous nephropathy transplanted in the donor kidney: observations of resolving glomerulopathy in serial allograft biopsies. Nephrol Dial Transplant. 2014;29(12):2343-2347. https://doi.org/10.1093/ndt/gfu333.
10. Pak JS, Deloughery ZJ, Wang J, et al. NELL2-Robo3 complex structure reveals mechanisms of receptor activation for axon guidance. Nat Commun. 2020;11(1):1489. https://doi.org/10.1038/s41467-020-15211-1.
11. Caza TN, Hassen SI, Dvanajscak Z, et al. NELL1 is a target antigen in malignancy-associated membranous nephropathy. Kidney Int. 2020;99(4):967-976. https://doi.org/10.1016/j.kint.2020.07.039.
12. Sprangers B, Lefkowitz GI, Cohen SD, et al. Beneficial effect of rituximab in the treatment of recurrent idiopathic membranous nephropathy after kidney transplantation. Clin J Am Soc Nephrol. 2010;5(5):790-797. https://doi.org/10.2215/CJN.04120609.
13. Stahl R, Hoxha E, Fechner K. PLA2R autoantibodies and recurrent membranous nephropathy after transplantation. N Engl J Med. 2010;363(5):496-498. https://doi.org/10.1056/NEJMc003066.
14. Gupta G, Fattah H, Ayalon R, et al. Pre-transplant phospholipase A2 receptor autoantibody concentration is associated with clinically significant recurrence of membranous nephropathy post-kidney transplantation. Clin Transplant. 2016;30(4):461-469. https://doi.org/10.1111/ctr.12711.
15. Chen P, Shi S-F, Qu Z, et al. Characteristics of patients with coexisting IgA nephropathy and membranous nephropathy. Ren Fail. 2018;40(1):213-218. https://doi.org/10.1080/0886022X.2018.1455591.
16. Cassol CA, Bott C, Nadasdy GM, et al. Immunostaining for galactose-deficient immunoglobulin A is not specific for primary immunoglobulin A nephropathy. Nephrol Dial Transplant. 2020;35(12):2123-2129. https://doi.org/10.1093/ndt/gfz152.
17. Zhao LU, Peng L, Yang D, et al. Immunostaining of galactose-deficient IgA1 by KM55 is not specific for immunoglobulin A nephropathy. Clin Immunol. 2020;217:108483. https://doi.org/10.1016/j.clim.2020.108483.
18. Lee M, Suzuki H, Kato R, et al. Renal pathological analysis using galactose-deficient IgA1-specific monoclonal antibody as a strong tool for differentiation of primary IgA nephropathy from secondary IgA nephropathy. CEN Case Rep. 2021;10(1):17-22. https://doi.org/10.1007/s13730-020-00508-3.
19. Dunckley T, Huentelman MJ, Craig DW, et al. Whole-genome analysis of sporadic amyotrophic lateral sclerosis. N Engl J Med. 2007;357(8):775-788. https://doi.org/10.1056/NEJMoa070174.
20. Habib GS, Saliba W, Nashashibi M, Armali Z. Penicillamine and nephrotic syndrome. Clin Transplant. 2007;357(8):775-788. https://doi.org/10.1056/NEJMoa070174.

SUPPORTING INFORMATION

Additional supporting information may be found in the Supporting Information section.

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