Netherlands
The Journal of Medicine

REVIEW

Anti-PLA$_2$R antibodies in membranous nephropathy: ready for routine clinical practice?

J.M. Hofstra*, J.F. Wetzels

Department of Nephrology, Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands,
*corresponding author: tel.:+ 31 (0)24-3614761, fax: +31 (0)24-3540022, e-mail: J.Hofstra@nier.umcn.nl

ABSTRACT

The identification of circulating autoantibodies against the M-type phospholipase A_2 receptor (anti-PLA$_2$R) in patients with idiopathic membranous nephropathy (iMN) has been a major discovery. Anti-PLA$_2$R can be measured by a commercially available test. It is suggested that measurement of anti-PLA$_2$R will change the diagnostic strategy in patients with nephrotic syndrome and may guide treatment in patients with iMN. We review the available evidence and caution against the immediate injudicious use of the assay in routine clinical practice.

KEYWORDS

Membranous nephropathy, anti-PLA$_2$R, nephrotic syndrome

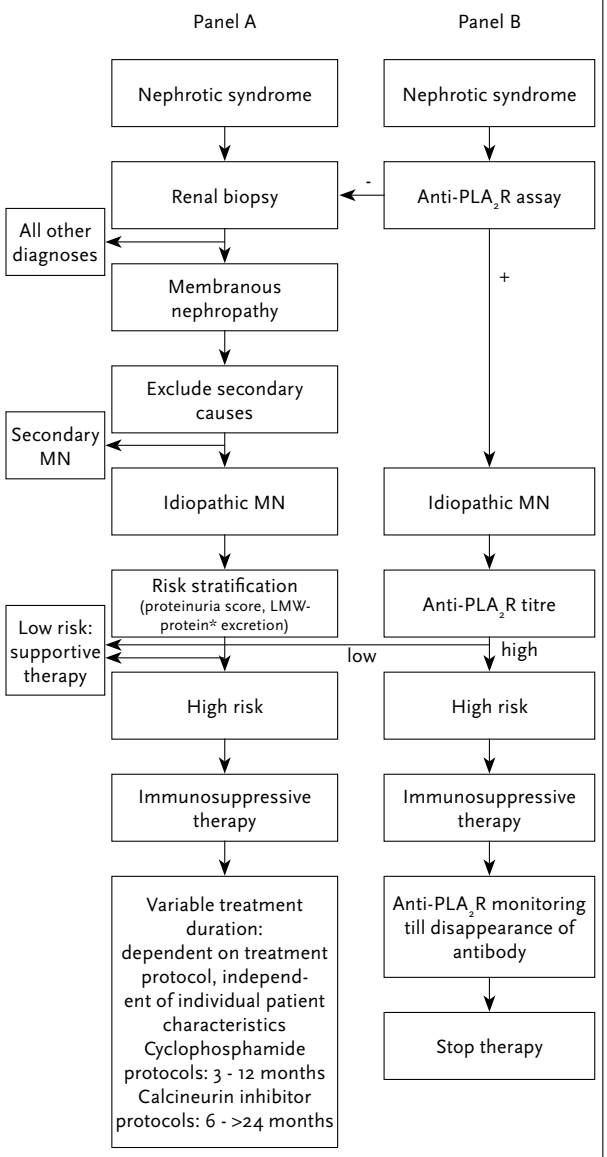
INTRODUCTION

Idiopathic membranous nephropathy (iMN) is the most common cause of nephrotic syndrome in the adult Caucasian population.[1] It is well established that iMN can develop due to the binding of a circulating antibody to an antigen that is present on podocytes.[2] In 2009 Beck *et al.* identified the M-type phospholipase A_2 receptor as an important antigenic target.[3] The authors showed that PLA$_2$R is expressed on podocytes and that antibodies against native PLA$_2$R, primarily of the IgG4 subclass, were present in the serum of approximately 70% of patients with iMN. This study provided the evidence that iMN is an autoimmune disease. The important role of PLA$_2$R in the pathogenesis was supported by the highly significant association between single nucleotide polymorphisms in the PLA$_2$R gene and the development of iMN.[4]
Membranous nephropathy can also develop secondary to systemic autoimmune diseases (SLE), infections (hepatitis B), drugs (NSAIDs), and malignancies. In these conditions the subepithelial deposits may arise from deposition of circulating immune complexes in the capillary wall or from binding of antibodies to antigens that are derived from the tumour and were planted in the basement membrane.[5]
Beck *et al.* already suggested that the detection of anti-PLA$_2$R in a patient with nephrotic syndrome may be pathognomonic for idiopathic MN, thus obviating the need for a diagnostic renal biopsy and an extensive search for underlying causes. Their findings and other recent data predicted that measurement of anti-PLA$_2$R may change the diagnostic algorithm in patients with nephrotic syndrome and guide treatment decisions in patients with iMN (*figure 1*).
Measurement of anti-PLA$_2$R is now possible with the development of an easy to use, commercially available assay. We briefly review the current evidence and express our view on the usefulness of this assay now and in the near future.

MEASUREMENT OF ANTI-PLA$_2$R ANTIBODIES: THE TECHNIQUES

Beck *et al.* employed a Western blot technique using glomerular extracts which were electrophoresed under nonreducing conditions.[3] Human serum was used as primary antibody, sheep antihuman IgG subclass antibodies as secondary antibodies, and finally peroxidase-labelled antisheep antibodies were used for detection. Quantitation was done by densitometric analysis and values expressed in arbitrary units. More recently an indirect immunofluorescence technique (IFT) was developed, which is now commercially available.[6] For this assay slides are made that contain biochips containing HEK 293 cells transfected with cDNA coding for PLA$_2$R and non-transfected cells as control. The biochips are incubated with human serum in different dilutions. The bound antibodies are detected

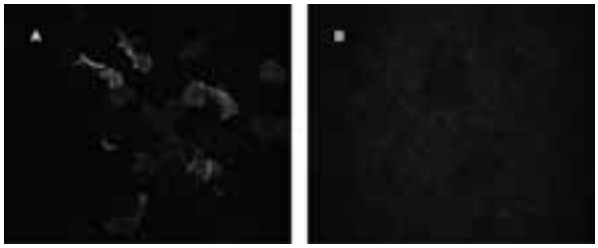
Figure 1. *Anticipated role of anti-PLA$_2$R antibody assay in the diagnosis and treatment of patients with nephrotic syndrome and membranous nephropathy*



Panel A illustrates the current algorithm for the diagnosis and treatment of idiopathic membranous nephropathy (adapted from Hofstra).[19] Panel B illustrates future practice if the ongoing prospective studies confirm the high specificity of anti-PLA2R for the diagnosis of idiopathic membranous nephropathy. We have also depicted the potential role of a quantitative assay in predicting risk of progression and determining duration of therapy. LMW-protein = low-molecular-weight protein; MN = membranous nephropathy.

with FITC-labelled goat antihuman IgG. The test result in an anti-PLA$_2$R-positive patient is depicted in *figure 2*. Quantitation can be performed by using different serum dilutions. UK investigators have developed an ELISA assay, which allows rapid and simple quantitation, but is only used for research purposes.[7] It is expected that commercial ELISA assays will become available in 2012. With such

Figure 2. *Indirect immunofluorescence test for anti-PLA$_2$R antibodies*



A: In a positive patient the antibodies bind to the PLA2R-positive HEK293 cells, leading to a positive immunofluorescence staining. B: The non-transfected PLA$_2$R-negative HEK293 cells serve as a control.

assays, quantitation will become simple and cost effective. Preliminary data suggest that there are large discrepancies between the quantitative results of the IFT and ELISA assays.

ANTI-PLA$_2$R IN IDIOPATHIC MEMBRANOUS NEPHROPATHY

In the pivotal study by Beck and coworkers, anti-PLA$_2$R antibodies were found in 70% of patients with iMN. Meanwhile, several studies have reported the prevalence of anti-PLA$_2$R-positive patients in different cohorts. These data are summarised in *table 1*. The percentage of anti-PLA$_2$R-positive patients ranges from 52% in a German study to 82% in a Chinese cohort.[6,8] These differences may be explained by differences in race, in the technique of the assay, or in the clinical characteristics. The study by Hoxha *et al.*, which reported the prevalence of 52%, was a cross-sectional study. These authors included patients with active as well as inactive disease. In 48% of patients no data on proteinuria were available. When limiting the analysis to patients with proteinuria >3.5 g/day, which likely reflects active disease, the percentage of patients with anti-PLA$_2$R antibodies was 66%. The Chinese group of Qin *et al.*, who reported an initial prevalence of 82%, repeated their assay in the negative patients, using less diluted patient serum and a higher concentration of detecting antibody. They observed a low titre of anti-PLA$_2$R antibody in 10 of 11 apparently negative patients. This study suggests that almost all patients with iMN may have detectable serum antibodies against PLA$_2$R.

ANTI-PLA$_2$R IN SECONDARY MEMBRANOUS NEPHROPATHY

Several authors have measured the presence of anti-PLA$_2$R antibodies in patients with secondary MN (*table 2*). It is obvious that the number of patients with secondary MN

Table 1. *Prevalence of anti-PLA$_2$R in idiopathic membranous nephropathy*

Author (year)	Patients (n)	aPLA$_2$R + (n; %)	Assay	Remarks
Beck (2009)[3]	37	26 (70)	WB	
Hofstra (2011)[17]	18	14 (78)	WB	
Beck (2011)[18]	35	25 (71)	WB	
Debiec (2011)[10]	42	24 (57)	IFT	
Hoxha (2011)[6]	100	52 (52)	IFT	Cross-sectional study; 66% positive if limiting analysis to patients with proteinuria >3.5 g/day
Qin (2011)[8]	60	49 (82)	WB	Low titres of PLA$_2$R present in 10/11 patients who were negative in standard assay Renal biopsies with mesangial or subendothelial deposits or glomerular infiltrating cells were excluded
Bruschi (2011)[20]	24	14 (58)	WB	Patient characteristics not provided
Beck (2011)[21]	14	12 (86)	WB	
Hoxha (2011)[11]	81	53 (65)	IFT	Prospective study
Schönermarck (2011)[9]	16	11 (69)	IFT	
Kanigicherla (2011)[7]	40	29 (73)	ELISA	Cross-sectional study; table reflects only patients with active disease

aPLA$_2$R + = anti-PLA$_2$R antibodies detectable; WB = Western blot; IFT = immunofluorescence test; ELISA = enzyme-linked immunosorbent assay.

included in the various studies is very low. Although most patients with secondary MN were negative for anti-PLA$_2$R antibodies, conflicting results have been reported. Of note, the authors suggest that the presence of anti-PLA$_2$R antibodies in patients with secondary MN might result from the co-incidental simultaneous development of iMN and the systemic disease, such as sarcoidosis or a malignancy. This suggestion was supported by Qin *et al.* who showed that in the PLA$_2$R-positive patients proteinuria persisted despite resection of the tumour.[8] These authors also showed that PLA$_2$R positivity paralleled the presence of IgG4 in the biopsy.

However, it is evident that more data are needed before we can safely conclude that the presence of anti-PLA$_2$R antibodies always reflects iMN and obviates the need to search for an underlying cause.

ANTI-PLA$_2$R IN PATIENTS WITHOUT MEMBRANOUS NEPHROPATHY

PLA$_2$R antibodies have not been detected in healthy controls (*table 3*). The antibodies were also absent in patients with proteinuria due to other glomerular diseases such as minimal change nephropathy, focal segmental glomerulosclerosis, or IgA nephropathy. However, the numbers in the literature are small. The total number of patients with non-membranous glomerular disease was 15 in Beck's study and 14 in a recent study by Schönermarck.[3,9] Hoxha studied 90 patients; however, only 18 had proteinuria >3.5 g/day.[6]

ANTI-PLA$_2$R ANTIBODIES IN MEMBRANOUS NEPHROPATHY: DISCORDANCE BETWEEN SERUM AND BIOPSY DATA

The discovery of anti-PLA$_2$R antibodies in serum of patients with MN and the presence of PLA$_2$R on the podocyte seemed to have solved the pathogenesis of MN. However, thus far there is no proof that the antibodies are pathogenic. There are no experimental models that are suited to study the pathogenesis of MN and the role of anti-PLA$_2$R since PLA$_2$R is not expressed in rat or mouse glomeruli. To study the pathogenicity of anti-PLA$_2$R antibodies the development of mice that stably overexpress PLA$_2$R in podocytes is required.

Recent studies further questioned the role of serum anti-PLA$_2$R as the main pathogenic antibody in iMN. Debiec *et al.* measured anti-PLA$_2$R in the serum of 42 patients with iMN and assessed the presence of

Table 2. *Prevalence of anti-PLA$_2$R antibodies in secondary membranous nephropathy*

Author	SLE		HBV/HCV		Malignancy		Other	
	N	aPLA$_2$R+	N	aPLA$_2$R+	N	aPLA$_2$R+	n	aPLA$_2$R+
Qin[8]	20	1	16	1	10	3	-	-
Hoxha[6]	6	0	1	0	3	0	7	0
Beck[3]	6	0	2	0	-	-	-	-
Knehtl[22]	-	-	-	-	-	-	1	1
Brenchley#	20	1			6	1		

SLE = systemic lupus erythematosus; HBV = hepatitis B virus; HCV = hepatitis C virus; aPLA$_2$R+ = anti-PLA$_2$R antibodies detectable; # personal communication ASN Kidney week 2011.

Table 3. *Prevalence of anti-PLA$_2$R antibodies in non-membranous nephropathy*

Author	Controls		Patients with glomerular disease		Remarks
	n	aPLA$_2$R+	n	aPLA$_2$R+	
Beck[3]	30	0	15	0	DN and FSGS
Qin[8]	20	0	-	-	
Hoxha[6#]	153	0	18	0	Most MCD n=10
Schönermarck[9]	-	-	14	0	Unknown

aPLA$_2$R + = anti-PLA$_2$R antibodies detectable; DN = diabetic nephropathy; FSGS = focal segmental glomerulosclerosis; MCD = minimal change disease; # analysis restricted to patients with proteinuria >3.5 g/day.

PLA$_2$R antigen in the renal biopsies.[10] Although there was concordance in the majority of patients, important exceptions were noted. In ten patients serum was negative whereas the renal biopsy was positive; the opposite occurred in three other patients. Hoxha also studied serum and biopsies in parallel.[11] PLA$_2$R expression was seen in renal biopsies of 47 patients, antibodies were found in 45 of them. In contrast, these authors did not report patients with detectable antibodies in serum, without expression of the antigen in the biopsy.

It is well know that iMN is an IgG4 dominant disease, with IgG4 being the dominant IgG subclass in renal biopsies of patients with iMN. Both Beck *et al.* and Qin *et al.* showed that antibodies against PLA$_2$R were mainly of the IgG4 subclass, confirming the dominance of IgG4.[3,8] Since IgG4 is not binding complement, this has sparked the debate on the pathogenic role of these antibodies. In a recent study published in abstract form it was suggested that PLA$_2$R IgG4 may bind mannose binding lectin (MBL), and thus activates complement via the MBL pathway.[12]

However, both Beck and Qin agree that other subclass specificities, particularly of the IgG1 and IgG3 subclass, can be found in most patients. The role of IgG4 is further questioned by the finding that IgG4 deposits in the biopsy do not always correlate with anti-PLA$_2$R titre in serum. Qu *et al.* did not observe IgG4 in 6/42 patients with iMN.[13] Hoxha reported three patients with anti-PLA$_2$R in serum and PLA$_2$R in the biopsy with negative IgG4 staining.[11]

ANTI-PLA$_2$R AND RECURRENCE OF IMN AFTER TRANSPLANTATION

In patients with iMN who develop end-stage renal disease, renal transplantation is the preferred therapy. Unfortunately, the post-transplant course is complicated by a recurrence of the disease in 10 to 45% of patients.[14] The discovery of anti-PLA$_2$R sparked studies that evaluated the role of these antibodies in developing and predicting recurrent disease. In a recent case report Stahl *et al.* described a patient with high anti-PLA$_2$R titres at the time of transplantation, who developed a recurrence almost immediately after transplantation.[15]

The potential role of anti-PLA$_2$R antibodies in recurrent membranous nephropathy was questioned by Debiec *et al.*[16] These authors reported ten kidney transplant recipients with iMN and recurrent disease and six patients with iMN without recurrence after transplantation. In six out of ten patients with recurrent disease PLA$_2$R was present in deposits in the native kidney biopsy, suggesting anti-PLA$_2$R-related disease. In five of those, anti-PLA$_2$R antibodies were detectable in serum and/or biopsy at the time of recurrence. Of note, in one patient there was recurrence in the graft, but anti-PLA$_2$R antibodies were not detectable. Moreover, the course was peculiar in two other patients. In one patient, serum antibodies were present at the time of transplantation. The anti-PLA$_2$R had disappeared at the time of recurrence, although the biopsy expressed PLA$_2$R in the deposits. The second patient had anti-PLA$_2$R antibodies in the serum, but no PLA$_2$R detectable in the biopsy.

On the other hand, in three out of six patients without recurrence, antibodies were present in the serum at the time of transplantation. Apparently, the presence of anti-PLA$_2$R antibodies did not lead to recurrent disease in these patients up till four years after transplantation. Clearly, more data are needed before firm conclusions can be made.

ANTI-PLA$_2$R AND TREATMENT OF MEMBRANOUS NEPHROPATHY

Beck *et al.* described the association between the presence of circulating anti-PLA$_2$R and clinical disease activity in eight patients with iMN.[3] In a subsequent collaborative study we studied 18 patients with nephrotic syndrome.[17] In 14 patients antibodies against PLA$_2$R were found. Antibody status was determined in serum samples obtained at baseline, during remission and during relapse. We observed a striking correlation between anti-PLA$_2$R titre and proteinuria, when using both baseline data and data from all time points. Moreover, anti-PLA$_2$R antibodies disappeared in all patients but one during remission, and reappeared in all evaluable patients during relapse. These studies were extended by the Mayo group.[18] These investigators evaluated the time course of anti-PLA$_2$R titres in relation to proteinuria and outcome in patients with iMN treated with rituximab. They observed that the decrease of anti-PLA$_2$R titre preceded the decrease of proteinuria, and predicted outcome.

Kanigichirla *et al.* measured anti-PLA_2R by ELISA. In their cross-sectional analysis antibody positivity was related to disease activity.[7] Antibodies were present in 29 of 40 patients with active disease (73%), compared with 15% positivity in patients in partial or complete remission. Moreover, the authors noted that in patients with active disease the titre of PLA_2R predicted outcome.

An association between anti-PLA_2R titres and outcome was also suggested by Qin *et al.* In patients with low titres time to remission was considerably shorter (6.6 *vs* 14.5 months), and likelihood of remission was higher (50 *vs* 30% at 12 months).[8]

CONCLUSIONS

The discovery of anti-PLA_2R antibodies in patients with idiopathic membranous nephropathy is of utmost importance. This finding has established iMN as an autoimmune disease and greatly stimulated research on its pathogenesis. It is expected that the measurement of anti-PLA_2R antibodies will improve our diagnostic and therapeutic strategy in patients with nephrotic syndrome in general and iMN in particular. However, there are some caveats. Conclusions so far are based on small, mostly retrospective studies. The accuracy of the test to identify iMN and to exclude secondary causes of MN awaits well-designed prospective studies, which are currently underway. We do not know if assays are comparable. It is expected that quantitation of the anti-PLA_2R antibodies will be important for prediction of prognosis as well as guidance of therapy. In this respect we are eagerly awaiting the development of an ELISA assay.

In our opinion it is too early to discard a renal biopsy in patients with nephrotic syndrome. Also, we advise to carefully check secondary causes using simple tools in all patients with MN.[19] Meanwhile, we advise to store serum samples taken at baseline and during follow-up. This would allow to perform measurements at a time point when questions regarding therapy efficacy or cause of relapsing proteinuria arise.

ACKNOWLEDGMENTS

We thank Dr. R.G. van der Molen, medical-immunologist and G.A.S. van de Wiel, senior technician, of the Department of Laboratory Medicine, Radboud University Nijmegen Medical Centre, for their valuable help with the introduction of the anti-PLA_2R assay. *Figure 2* was kindly provided by Ms. van de Wiel. The author is supported by a grant of the Dutch Kidney Foundation (KJPB 11.021).

REFERENCES

1. Tiebosch AT, Wolters J, Frederik PF, et al. Epidemiology of idiopathic glomerular disease: a prospective study. Kidney Int. 1987;32(1):112-6.
2. Debiec H, Ronco P. Fetomaternal alloimmunization with antenatal glomerulopathies. Ann N Y Acad Sci. 2007;1110:559-66.
3. Beck LH, Bonegio RG, Lambeau G, et al. M-type phospholipase A2 receptor as target antigen in idiopathic membranous nephropathy. N Engl J Med. 2009;361(1):11-21.
4. Stanescu HC, Arcos-Burgos M, Medlar A, et al. Risk HLA-DQA1 and PLA(2)R1 alleles in idiopathic membranous nephropathy. N Engl J Med. 2011; 364(7):616-26.
5. Glassock RJ. Human idiopathic membranous nephropathy--a mystery solved? N Engl J Med. 2009;361(1):81-3.
6. Hoxha E, Harendza S, Zahner G, et al. An immunofluorescence test for phospholipase-A-receptor antibodies and its clinical usefulness in patients with membranous glomerulonephritis. Nephrol Dial Transplant. 2011;26(8):2526-32.
7. Kanigicherla DKA, Brenchley PE, Venning M, et al. Association of Anti-PLA2R with Disease Activity and Outcome in Idiopathic Membranous Nephropathy [Abstract]. J Am Soc Nephrol. 2011;22:33A.
8. Qin W, Beck LH, Jr., Zeng C, et al. Anti-phospholipase A2 receptor antibody in membranous nephropathy. J Am Soc Nephrol. 2011;22(6):1137-43.
9. Schönermarck U, Eichhorn P, Sitter T, et al. Performance of Anti-Phospholipase A2-Receptor Antibody Testing for Membranous Nephropathy in Routine Clinical Practice [Abstract]. J Am Soc Nephrol. 2011;22:775A.
10. Debiec H, Ronco P. PLA2R autoantibodies and PLA2R glomerular deposits in membranous nephropathy. N Engl J Med. 2011;364(7):689-90.
11. Hoxha E, Zahner G, Velden J, et al. Phospholipase A2 Receptor Autoantibodies (PLA2R-AB) and Increased PLA2R Expression in Glomeruli Discriminate Primary from Secondary Membranous Nephropathy (MN) [Abstract]. J Am Soc Nephrol. 2011;22:68A.
12. Ma H, Beck LH, Salant DJ. Membranous Nephropathy-Associated Anti-Phospholipase A2 Receptor IgG4 Autoantibodies Activate the Lectin Complement Pathway [Abstract]. J Am Soc Nephrol. 2011;22:62A.
13. Qu Z, Liu G, Li J, et al. Absence of glomerular IgG4 deposition in patients with membranous nephropathy may indicate malignancy. Nephrol Dial Transplant 2011; doi: 10.1093/ndt/gfr534.
14. Choy BY, Chan TM, Lai KN. Recurrent glomerulonephritis after kidney transplantation. Am J Transplant. 2006;6(11):2535-42.
15. Stahl R, Hoxha E, Fechner K. PLA2R autoantibodies and recurrent membranous nephropathy after transplantation. N Engl J Med. 2010;363(5):496-8.
16. Debiec H, Martin L, Jouanneau C, et al. Autoantibodies specific for the phospholipase A2 receptor in recurrent and De Novo membranous nephropathy. Am J Transplant. 2011;11(10):2144-52.
17. Hofstra JM, Beck LH, Beck DM, et al. Anti-phospholipase A receptor antibodies correlate with clinical status in idiopathic membranous nephropathy. Clin J Am Soc Nephrol. 2011;6(6):1286-91.
18. Beck LH, Fervenza FC, Beck DM, et al. Rituximab-induced depletion of anti-PLA2R autoantibodies predicts response in membranous nephropathy. J Am Soc Nephrol. 2011;22(8):1543-50.
19. Hofstra JM, Wetzels JF. Management of patients with membranous nephropathy. Nephrol Dial Transplant. 2011; doi: 10.1093/ndt/gfr371.
20. Bruschi M, Carnevali ML, Murtas C, et al. Direct characterization of target podocyte antigens and auto-antibodies in human membranous glomerulonephritis: Alfa-enolase and borderline antigens. J Proteomics. 2011 Sep 6;74(10):2008-17.
21. Beck LH, Jr., Fervenza FC, Bomback AS, et al. Response of Anti-PLA2R to Adrenocorticotropic Hormone (ACTH) Gel in Membranous Nephropathy [Abstract]. J Am Soc Nephrol. 2011;22:33A.
22. Knehtl M, Debiec H, Kamgang P, et al. A case of phospholipase A receptor-positive membranous nephropathy preceding sarcoid-associated granulomatous tubulointerstitial nephritis. Am J Kidney Dis. 2011;57(1):140-3.