

Original Article

Prediction of membranous nephropathy recurrence after transplantation by monitoring of anti-PLA2R1 (M-type phospholipase A2 receptor) autoantibodies: a case series of 15 patients

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ABSTRACT

Background. The predictive value of anti-M-type phospholipase A2 receptor (PLA2R1) autoantibodies for membranous nephropathy (MN) recurrence after renal transplantation remains controversial.

Methods. Our aim was to monitor anti-PLA2R1 IgG4 activity using a sensitive enzyme-linked immunosorbent assay in 15 kidney transplant recipients with MN, and to test the correlation between antibody titres and MN recurrence.

Results. Five patients never exhibited anti-PLA2R1 antibodies, and one of them relapsed. Ten patients (67%) had IgG4 anti-PLA2R1 antibodies at the time of transplantation and during follow-up. The presence of IgG4 anti-PLA2R1 antibodies at the time of kidney transplantation does not imply MN recurrence ($P = 0.600$, $n = 15$). However, a positive IgG4 anti-PLA2R1 activity during follow-up (>6 months) was a significant risk factor for MN relapse ($P = 0.0048$, $n = 10$). Indeed, four patients had persistent IgG4 anti-PLA2R1 activity after transplantation and relapsed. Among them, one was successfully treated with rituximab. Another had persistently high IgG4 anti-PLA2R1 activity and exhibited a histological relapse but no proteinuria while on treatment with renin-angiotensin system inhibitors. In contrast, the six other patients who did

not relapse exhibited a decrease of their IgG4 anti-PLA2R1 activity following transplant immunosuppression, including two with proteinuria due to biopsy-proven differential diagnoses. A weak transplant immunosuppressive regimen was also a risk factor of MN recurrence ($P = 0.0048$, $n = 10$). Indeed, the six patients who received both an induction therapy and a combined treatment with calcineurin inhibitors/mycophenolate exhibited a decrease of IgG4 anti-PLA2R1 activity and did not relapse, while the four patients who did not receive this strong immunosuppressive treatment association had persistently high IgG4 anti-PLA2R1 activity and relapsed.

Conclusion. The monitoring of IgG4 anti-PLA2R1 titres during follow-up helps to predict MN recurrence, and a strong immunosuppressive treatment of anti-PLA2R1 positive patients may prevent recurrence.

Keywords: membranous nephropathy, PLA2R1 autoantibodies, prognosis, recurrence, transplantation

INTRODUCTION

Membranous nephropathy (MN) is a common cause of nephrotic syndrome in adults and leads, in 40–50% of cases, to end-stage kidney disease (ESKD) [1–4]. MN represents 2% of

patients on the waiting list for a kidney graft [5, 6]. Recurrence of MN after renal transplantation occurs in 7–42% of patients [5, 7], resulting in reduced allograft survival [6, 8]. However, the true proportion of MN recurrence is difficult to assess because graft biopsy policies are extremely variable among transplant centres. The highest MN recurrence rate is found in series of systematic protocol biopsies [5]. Furthermore, the type of immunosuppressive treatment is not yet recognized as a determinant of MN recurrence [9].

MN is defined by subepithelial immune deposits containing IgG (mainly of the IgG4 subclass) and complement fractions with alteration of the basement membrane structure. The M-type phospholipase A2 receptor (PLA2R1) is the major autoantigen involved in MN [10]. This has been widely confirmed in patients with idiopathic or secondary MN using different assays including western blot, indirect immunofluorescence transmission (IIFT) on human embryonic kidney (HEK) cells transfected with PLA2R1 and enzyme-linked immunosorbent assay (ELISA) [11–14]. The pathogenic role of anti-PLA2R1 autoantibodies is not yet proven, but their serum titres measured by western blot [12], IIFT [15] or ELISA with recombinant human PLA2R1 [13, 14] appear to be associated with MN disease activity in native kidneys. Indeed, the autoantibody titres were found to correlate with baseline proteinuria and the rate of remission [13, 15]. Furthermore, the disappearance of anti-PLA2R1 antibodies precedes proteinuria decrease and disease remission upon treatment with rituximab [16]. Moreover, the antibody titre may also predict long-term outcome in MN patients [17, 18]. However, only partial or no correlation between anti-PLA2R1 activity and clinical parameters were observed in some studies [13, 19, 20]. Whether these discrepancies are due to the detection methods used and/or the series of MN patients analysed remains to be clarified.

The relevance of anti-PLA2R1 antibody monitoring for the prediction of MN recurrence in kidney transplant recipients also remains controversial. Stahl *et al.* [21] first reported a case of MN recurrence after renal transplantation. Anti-PLA2R1 antibody titres on IIFT increased before proteinuria and decreased after four boluses of rituximab that induced remission [21]. Controversy arises from a study of 10 patients with recurrent MN, suggesting that anti-PLA2R1 antibodies were implicated in only 5 of 10 patients, with no correlation between the presence of the autoantibodies detected by IIFT and disease recurrence [22]. However, several patients had no sera available at the time of either renal transplantation and/or MN recurrence. Furthermore, MN did not recur in three patients who had high titres of anti-PLA2R1 antibodies at the time of renal transplantation, but subsequent testing during follow-up was not reported [22].

The aim of this study was to monitor anti-PLA2R1 antibody titres during the follow-up of 15 kidney transplant recipients with MN and to correlate the autoantibody titres with disease activity on kidney graft using an anti-PLA2R1-specific ELISA.

MATERIALS AND METHODS

Patients

Fifteen consecutive MN patients, who received a kidney transplant between 1997 and 2012 at the University Hospital

of Nice, with at least a 1-year follow-up, were included in this study. All patients had MN diagnosis on native kidney established by renal biopsy. Patients gave informed consent to participate in this study, which was approved by our local research ethical committee. Primary MN was defined for patients, where no secondary cause was apparent. Secondary MN was suggested by the presence of anti-nuclear antibodies, hepatitis B or C serologies or cancers, but a clear classification into secondary MN was made only when the secondary cause or its treatment was associated with an increase or decrease of MN disease (see the ‘Results’ section for Patients 7, 10, 11 and 14 who were classified as I or II MN based on their clinical data). MN recurrence was established on protocol biopsies at Month 3 and/or Year 1, or on biopsies performed because of clinical or biological events. MN recurrence was established by immunofluorescence showing typical IgG subepithelial deposits in a granular pattern. Sera were analysed at the time of renal transplantation and during follow-up.

Detection of anti-PLA2R1 antibodies by ELISA

We developed a novel ELISA to specifically and quantitatively measure anti-PLA2R1 antibody titres in MN patients (Seitz-Polski *et al.*, manuscript in preparation). Briefly, a recombinant soluble form of PLA2R1 encompassing the entire extracellular region of the receptor was produced and purified from transfected HEK 293 cells as described previously [23–25]. The purified antigen (1 µg/mL) was then used to coat ELISA plates at 4°C overnight. Plates were blocked for 2 h with SeramunBlock (Seramun Diagnostica, Germany). Patients’ sera were diluted at 1:100 in phosphate-buffered saline (PBS) plus 0.1% low-fat dry milk. After 2 h of incubation at room temperature on a plate shaker, the plates were washed four times with PBS plus 0.02% Tween 20. Anti-human IgG4-horseradish peroxidase conjugate (Southern Biotech) diluted at 1:7500 in SeramunStab ST plus (Seramun Diagnostica), or anti-human total IgG-horseradish peroxidase conjugate [14] was added (100 µL per well) and incubated for 1 h at room temperature on a plate shaker. After four washes, the tetramethylbenzidine peroxidase substrate was added for 15 min, after which the reaction was stopped with HCl 1.2 N. The plates were read at 450 nm.

The anti-PLA2R1 ELISA was set to detect either total IgG or IgG4 and was validated on a cohort of 153 MN patients (130 idiopathic MN and 23 secondary MN) versus 134 control subjects (67 healthy donors and 67 disease controls). The specificity of the ELISA was 100% and was also confirmed by western blot and IIFT assays (Euroimmun kit) as described [10, 12]. The IgG4 ELISA was found to be the most sensitive test for patients with active MN with an average positivity in MN patients (both idiopathic and secondary forms) of 64% versus only 58% for IIFT and 57% for total IgG ELISA (Table 1). All human sera used in this retrospective study were assayed in duplicates at a working dilution of 1:100 for both IgG4 and total IgG anti-PLA2R1 activity. Because IgG4 was the dominant subclass in the majority of patients and IgG4 titres were found to significantly correlate with disease activity ([11–14] and Seitz-Polski *et al.*, manuscript in preparation), IgG4 anti-PLA2R1 titres are presented during the patient’s follow-up. A positive index patient serum was used to generate a standard IgG4 anti-PLA2R1

Table 1. Patient's baseline clinical characteristics, immunosuppressive treatment and anti-PLA2R1 titres before and after kidney transplantation

Patients	Age ^a	Sex	MN	Day of transplantation				MN recurrence				Last observation carried forward						
				Anti-PLA2R1 levels			Immunosuppressive treatment	Yes/ no	Time from Tx to recurrence (months)	Anti-PLA2R1 levels			Follow-up	UPCR (g/g)	sCreat (μmol/L)	Anti-PLA2R1 levels		
				ELISA IgG4 ^b	ELISA Tot IgG ^b	IIFT Tot IgG				ELISA IgG4 ^b	ELISA Tot IgG ^b	IIFT Tot IgG				ELISA IgG4 ^b	ELISA totIgG ^b	IIFT Tot IgG
1	32	M	I	1321	918	1/100	Cyclo, MMF	Yes	12	NA	NA	NA	192	0.21	143	305	N	1/32
2	48	F	I	166	787	1/10	Cyclo, Rapa	Yes	156	5023	3000	1/320	162	7.06	117	217	137	1/10
3	54	M	I	7364	1561	1/1000	Thymoglobulin (Thymo), Tacro, MMF	No ^c	-	N	N	N	186	0.62	277	N	N	N
4	59	M	I	5084	1282	1/320	Basiliximab, Tacro, Evero	Yes	8	307	N	1/32	84	0.22	201	N	N	N
5	57	M	I	1261	1195	1/100	Tacro, MMF	Yes	60	633	605	1/32	60	3.69	114	633	605	1/32
6	62	F	I	137	N	N	Basiliximab, Tacro, MMF	No ^c		N	N	N	54	1.13	247	N	N	N
7	64	F	I (HBV) ^d	187	140	1/10	Basiliximab, Tacro, MMF	No ^e		N	N	N	42	0.04	93	N	N	N
8	52	F	I	5014	1190	1/10	Basiliximab, Tacro, mycophenolate acid	No ^c		N	N	N	36	0.50	90	N	N	N
9	42	M	I	1129	772	1/100	Basiliximab, Tacro, MMF	No ^f		N	N	N	24	0.09	96	N	N	N
10	58	M	I (HBV, HCV) ^d	3160	1557	1/320	Basiliximab, Tacro, MMF	No ^f		N	N	N	24	0.50	90	N	N	N
11	35	F	II (SLE) ^d	N	N	N	Tacro, MMF	No ^f		N	N	N	168	0.06	130	N	N	N
12	39	M	I	N	N	N	Tacro, MMF	No ^c		N	N	N	54	0.08	140	N	N	N
13	60	F	I	N	N	N	Thymo, Tacro, MMF	Yes	3	N	N	N	12	HD	HD	N	N	N
14	58	M	II (HCV) ^d	N	N	N	Thymo, Tacro, MMF	No ^e		N	N	N	18	0.37	187	N	N	N
15	70	H	I	N	N	N	Basiliximab, Tacro, MMF	No ^f		N	N	N	10	0.20	138	N	N	N

M, male; F, female; MN, membranous nephropathy (I, primary or II, secondary); totIgG, total IgG; HCV/HBV, hepatitis C/B virus; SLE, systemic lupus erythematosus; ESKD, end-stage kidney disease; NA, non-available; N, negative during follow-up; Tx, transplantation; IIFT, indirect immunofluorescence transmission; Cyclo, Cyclosporine; Tacro, tacrolimus; Evero, everolimus; MMF, mycophenolate mofetil; sCreat, serum creatinine; HD, haemodialysis.

^aAt the time of renal transplantation.

^bRU/mL.

^cPatients 3, 6, and 14 had kidney graft biopsy that showed no MN recurrence but differential diagnosis.

^dPatients 7 and 10 have I MN with incidental HBV and/or HCV infection while Patients 11 and 14 have II MN (see the main text).

^ePatients 7, 8 and 12 had protocol biopsies that showed no MN recurrence.

^fPatients 9, 10, 11 and 15 never had kidney graft biopsy because their UPCR remained <0.5 g/g during follow-up without RAS blockade.

calibration curve and to convert OD_{450 nm} values into relative units/mL (RU/mL). Sixty-seven serum samples from healthy donors were used to define the normal range, below the mean value + 3 SDs; values >128 RU/mL were considered as positive in the IgG4 anti-PLA2R1 ELISA.

Detection of anti-PLA2R1 antibodies by IIFT

We used a recombinant cell-based indirect immunofluorescence test (RC-IFA; Euroimmun, Germany) containing a BIOCHIP mosaic of formalin-fixed HEK 293 cell over-expressing human PLA2R1 and mock-transfected HEK 293 cells as negative control [15]. To achieve a semi-quantitative measurement of PLA2R1 autoantibody levels, different dilutions of serum were prepared in PBS, 0.2% Tween 20 and incubated for 30 min. A fluorescein isothiocyanate-conjugated goat anti-human IgG antibody (Euroimmun) was used to detect bound IgG antibodies. All slides were evaluated by two independent observers using a microscope with 460–490 nm LED excitation (EUROStar; Euroimmun). A specific fluorescence of the transfected cells at a dilution of 1:10 or higher was considered positive.

Statistical analyses

For descriptive statistics, data are presented as mean ± SD. Qualitative criteria were compared using Fisher's exact test. Quantitative variables were compared using the Student *t*-test. All statistics were performed using the Prism6 software. P-values of <0.05 were considered as statistically significant.

RESULTS

Patients

The 15 patients included in this study are described in Table 1. They were nine males and six females. Their mean age was 53 years (32–64 years) at the time of renal transplantation. Eleven patients presented idiopathic MN with no apparent secondary cause. Patients 7, 10, 11 and 14 had features of a secondary form: one to systemic lupus erythematosus (SLE), one to hepatitis B virus (HBV) infection, one to hepatitis C virus (HCV) infection and one to both HBV and HCV infection. However, Patients 7 and 10 had likely idiopathic MN with incidental HBV and/or HCV infection and not secondary MN. Indeed, no therapeutic association was found between HBV/HCV infection and MN disease activity. Clinically, Patient 7 was successfully treated for HBV infection but developed kidney failure while on HBV treatment. Patient 10 had both HBV and HCV infection that clinically improved spontaneously while MN disease became more severe and progressed to ESKD. These clinical data support the view of two independent diseases, i.e. idiopathic MN and HBV/HCV infection. This view is reinforced by the fact that the sole presence of anti-PLA2R1 autoantibodies in MN patients is a sufficient criterion to classify these latter as primary MN [19]. Because both Patients 7 and 10 were positive for anti-PLA2R1 (see below), we have classified these patients as idiopathic MN with incidental HBV/HCV infection. Patient 11 had MN and active SLE. She was clearly positive for anti-DNA antibody and was treated with corticosteroids and antimalarial drugs

over 10 years without remission while she developed ESKD and underwent renal transplantation. Patient 14 had MN and chronic HCV infection before and after the first kidney transplantation. Seventeen years later, he lost the kidney graft (without MN recurrence) and was successfully treated for HCV before the second kidney transplantation. Furthermore, Patients 11 and 14 were negative for anti-PLA2R1 antibodies (see below). For both patients, it is thus unclear whether they have primary MN (here with an unknown antigen–antibody pair, distinct from PLA2R1) together with incidental SLE or HCV infection or a true secondary MN. In the absence of more clinical evidence, we have maintained the classification of Patients 11 and 14 as secondary MN. Seven patients received a specific treatment for MN on their native kidney: one was treated with cyclosporine and mycophenolate mofetil (MMF), one with cyclosporine and steroids, one with steroids alone, one for SLE with steroids and hydroxychloroquine and two for HBV or HCV infection by baraclud or combined interferon and ribavirin. The mean time from diagnosis of MN to ESKD was 7 years (7 months to 17 years). All but one patient was on dialysis before kidney transplantation for a mean time of 26 months (0–11 years).

All patients received a deceased donor kidney. Ten of them had an induction of immunosuppression with thymoglobulins (three cases) or anti-CD25 monoclonal antibodies (seven cases). Subsequent immunosuppressive treatments are detailed in Table 1. The mean follow-up was 74 months (12 months–16 years). Four patients (9, 10, 11 and 15) never had kidney graft biopsy since urinary protein–creatinine ratio (UPCR) remained <0.5 g/g during follow-up without renin–angiotensin system (RAS) blockade. Three patients (7, 8 and 12) had protocol biopsies at Month 3 (*n* = 1) or at Month 3 and Year 1 (*n* = 2). The last eight patients (1–6, 13 and 14) had biopsies because of serum creatinine increase or nephrotic range proteinuria (Table 1). Five patients (33%) presented a biopsy-proven MN recurrence on their renal transplant either early before Year 1 in three cases (Patient 1 at Year 1, Patient 4 at Month 8 and Patient 13 at Month 3) or lately after Year 5 in two other cases (Patient 2 at Year 13 and Patient 5 at Year 5). Only Patient 13 reached ESKD.

Detection of anti-PLA2R1 antibodies in patients

We used a validated ELISA to monitor both IgG4 and total IgG anti-PLA2R1 autoantibody titres (see Materials and Methods). Ten of the 15 patients (67%) had IgG4 anti-PLA2R1 activity at the time of renal transplantation and/or during follow-up (Table 1). Nine of them were also positive for total IgG anti-PLA2R1 activity. Patient 6 had only a borderline IgG4 but no total IgG anti-PLA2R1 activity. The 10 patients positive by ELISA were also positive by IIFT (Table 1). Because Patients 7 and 10 were classified as idiopathic MN but with incidental HBV/HCV infection and because Patient 7 had a low anti-PLA2R1 titre by ELISA and IIFT at the time of transplantation, we confirmed that these patients were also positive for anti-PLA2R1 by western blot [10] using purified recombinant human PLA2R1 antigen (Supplementary data, Figure S1). Finally, five patients had no anti-PLA2R1 antibodies at the time of renal transplantation and during follow-up.

Overall, 67% of all patients were positive for anti-PLA2R1. Among the idiopathic cases, 77% were positive. All patients positive for anti-PLA2R1 had idiopathic MN, with two of them having incidental HBV/HCV infection. The two cases of secondary MN were negative.

Monitoring of anti-PLA2R1 autoantibodies after kidney transplantation

Four patients (1, 2, 4 and 5) exhibited MN recurrence on their kidney graft and had persistent IgG4 anti-PLA2R1 activity at Month 6 or later (Table 1 and Figure 1 for Patients 1 and

4). For Patients 1 and 2, we performed PLA2R1 antigen staining on the biopsy [19] at the time of MN recurrence and found an enhanced staining for Patient 2, but only a faint staining for Patient 1 (Supplementary data, Figure S2). Enhanced PLA2R1 staining has been observed in most cases of primary MN [19], but discrepant results were obtained by Debiec *et al.* [22] in kidney transplant recipients with MN. Patient 1 had a sub-clinical MN recurrence with high IgG4 anti-PLA2R1 titre. Patients 2 and 5 had MN recurrence with increased proteinuria and persistent IgG4 anti-PLA2R1 titre. Patient 4 had positive IgG4, but no total IgG anti-PLA2R1 activity measured by

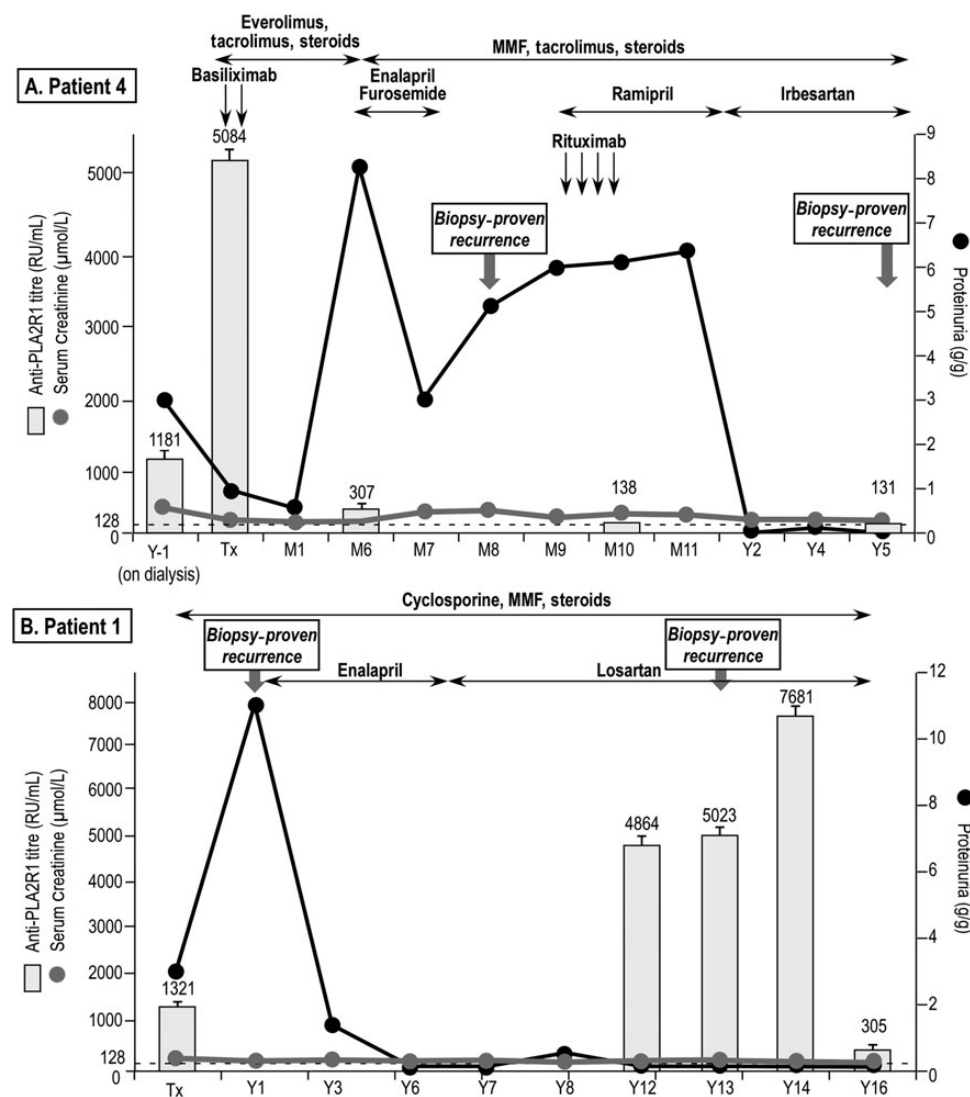


FIGURE 1: Clinical follow-up of two patients with recurrent MN. (A) Patient 4 had high IgG4 anti-PLA2R1 antibody titres on the day of renal transplantation. Anti-PLA2R1 activity remained significant at Month 6 (307 RU/mL), while he developed a nephrotic syndrome complicated by an intestinal ischaemia secondary to mesenteric thrombosis requiring anticoagulant. A kidney biopsy could only be performed after anticoagulant discontinuation at Month 8 and confirmed MN recurrence. He received four boluses of rituximab (375 mg/m²/week) with a complete disappearance of anti-PLA2R1 antibodies (138 RU/mL). Five years later, anti-PLA2R1 antibodies remain undetectable (131 RU/mL) with no proteinuria, but renal function has only marginally recovered [Modification of Diet in Renal Disease (MDRD) 26 mL/min/1.73 m²] despite the total disappearance of subepithelial IgG deposits. (B) Patient 1 presented high titres of IgG4 anti-PLA2R1 antibodies on the day of renal transplantation and during follow-up and exhibited a nephrotic syndrome and MN recurrence 1 year after transplantation. RAS blockade combined with increased dosage of steroids and high doses of cyclosporine and MMF allowed a complete clinical remission with a sequel of chronic renal failure (MDRD 45 mL/min/1.73 m²). However, 13 years later, he still has high titres of anti-PLA2R1 antibodies with IgG deposits on kidney biopsy.

ELISA at the time of MN recurrence with increased proteinuria. Patient 4 was successfully treated with rituximab and exhibited no anti-PLA2R1 activity at last observation with clinical and histological remission.

Six patients (3 and 6–10) did not relapse and experienced a decrease of their IgG4 anti-PLA2R1 activity during follow-up (Table 1 and Figure 2 for Patients 8 and 9). The minimum follow-up for these six patients was 24 months. Patients 3 and 6 exhibited an increase of proteinuria during follow-up without any associated anti-PLA2R1 activity. Kidney biopsy showed a differential diagnosis: BK nephropathy for Patient 3 and nephrosclerosis of an extended criteria kidney graft for Patient 6 (i.e. without evidence of MN recurrence).

The patients' cases reported in this study can be recapitulated in a working diagnostic tree using IgG4 anti-PLA2R1 activity and changes in proteinuria levels at the time of renal transplantation and during follow-up (Figure 3). Among the 10 patients with anti-PLA2R1 activity at the time of renal transplantation, the persistence of anti-PLA2R1 antibodies with high proteinuria levels is associated with overt MN recurrence ($n = 3$) while the persistence of anti-PLA2R1 activity with low proteinuria levels

under RAS blockade is associated with sub-clinical MN recurrence ($n = 1$). A decrease of IgG4 anti-PLA2R1 antibody titres with an increase of proteinuria levels is associated with a differential diagnosis ($n = 2$), and there is no MN recurrence when anti-PLA2R1 activity disappears and proteinuria decreases during follow-up ($n = 4$). Among the five patients who never presented any anti-PLA2R1 activity, Patient 13 had MN recurrence, possibly due to another antigen target. We carefully tested the sera of this patient by western blot for its reactivity against possible antigens which might be present in protein lysates from human kidney glomerular extract [10] and cultured human podocytes [20, 26, 27]. We tested three different sera of Patient 13 at high concentration (1/50) under both reducing and non-reducing conditions. The three sera were collected at the time of transplantation, at recurrence and during ESKD. In all conditions, no specific signal was obtained on human glomerular extracts (data not shown), even after detection with an ultra-sensitive enhanced chemiluminescence detection system. Using protein extract from human cultured podocytes, no specific signal was also observed when compared with results obtained with control sera either from MN patients positive for

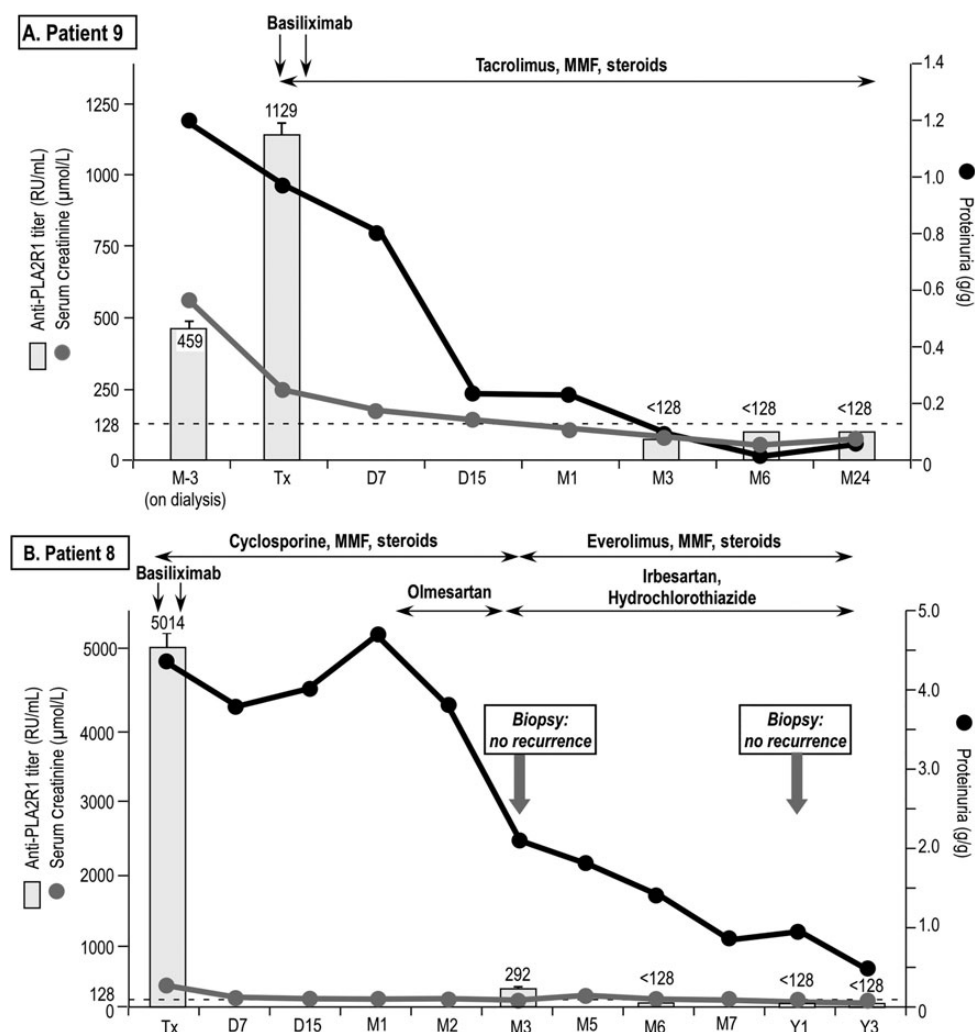


FIGURE 2: Clinical follow-up of two patients without MN recurrence. (A) Patient 9 exhibited a rapid disappearance of IgG4 anti-PLA2R1 activity followed by a decrease of proteinuria. (B) Patient 8 had a relatively slower decrease of IgG4 anti-PLA2R1 antibodies and proteinuria. Kidney biopsies ruled out MN recurrence at Months 3 and 12.

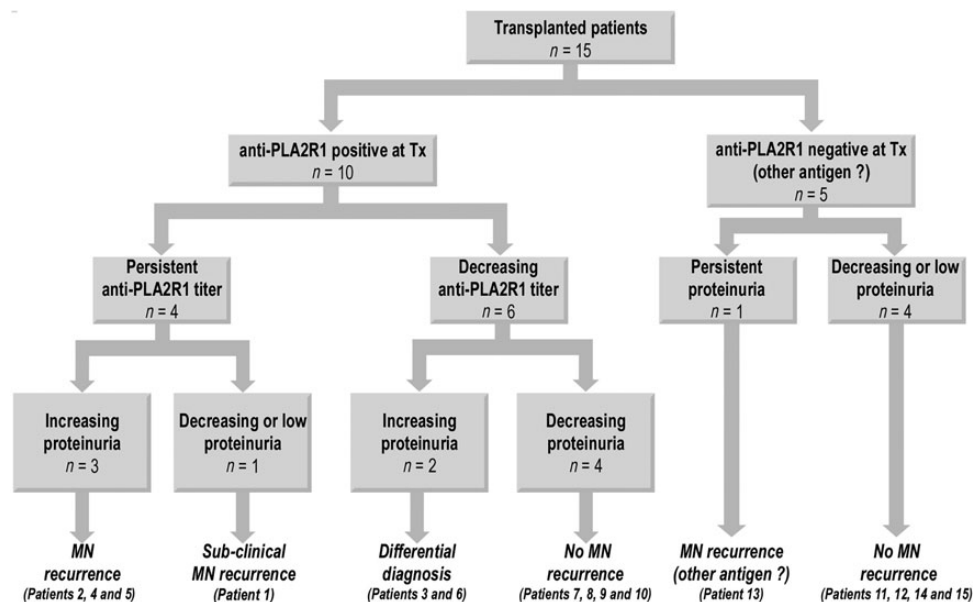


FIGURE 3: Working diagnostic tree using IgG4 anti-PLA2R1 activity at the time of renal transplantation and during follow-up, and proteinuria changes after renal transplantation.

PLA2R1 (which were indeed positive for PLA2R1 on this podocyte protein extract, see Supplementary data, Figure S3) or from a patient with focal segmental glomerulosclerosis or from a healthy donor (Supplementary data, Figure S3). Of interest, no specific signal was observed at molecular masses corresponding to those of aldose reductase, superoxide dismutase 2 and alpha-enolase, which have been proposed as autoantigens in MN [20, 27]. These results suggest that the serum of this patient has no or very limited amount of autoantibodies directed against an antigen present in human kidney glomeruli or cultured podocytes. Alternatively, the antigen of this patient might be a planted one that cannot be detected with these western blot assays. The identification of the putative antigen for Patient 13 should thus deserve future work.

Together, our results indicate that the presence of anti-PLA2R1 autoantibodies at the time of renal transplantation did not imply MN recurrence ($P = 0.600$, $n = 15$) (Table 1). However, a persistent IgG4 anti-PLA2R1 activity after 6 months of follow-up or more was significantly associated with MN recurrence ($P = 0.0048$, $n = 10$) and the mean IgG4 anti-PLA2R1 antibody titre after 6 months of follow-up was significantly higher in the group of patients with MN recurrence ($P < 0.0001$, $n = 10$) (Table 2). Moreover, none of the six patients who received both an induction therapy and combined calcineurin inhibitor (CNI) and mycophenolate relapsed, but all four patients who did not receive such strong immunosuppressive association relapsed ($P = 0.0048$) (Tables 1 and 2).

DISCUSSION

The relevance of monitoring anti-PLA2R1 antibody to predict MN recurrence in kidney transplant recipients has remained unclear [21, 22]. Therefore, the primary aim of this study was to further assess whether the monitoring of anti-PLA2R1

Table 2. Immunosuppressive treatment and anti-PLA2R1 activity in patients with and without MN recurrence

Patients	Recurrent MN		Non-recurrent MN	
	n	Anti-PLA2R1 activity during follow-up (RU/mL) ^a	n	Anti-PLA2R1 activity during follow-up (RU/mL)
Induction therapy and combined CNI and MMF	0*	–	6*	<128
No induction therapy or no CNI or no MMF	4*	1544 ± 2326	0*	–
Total population	4	1544 ± 2326**	6	<128**

^aMean anti-PLA2R1 titre at MN recurrence. The mean value was calculated from individual titres of Patients 1, 2, 4 and 5.

* $P = 0.0048$ for MN recurrence in patients who received both an induction therapy and combined CNI and MMF versus those who did not receive this strong immunosuppressive regimen.

** $P < 0.0001$ for anti-PLA2R1 levels during follow-up between patients with and without MN recurrence.

autoantibody may help or not to predict MN recurrence after transplantation. Similar to previous studies [21, 22], our study was retrospective and included a relatively small number of patients (15 cases). Interestingly enough, we found a comparable percentage of patients with anti-PLA2R1 activity (67%) and MN recurrence (33%). One major strength of our study is, however, that it is the first series of sequential analysis of anti-PLA2R1 antibody titres measured on sera available not only at the time of renal transplantation but also during follow-up and by measuring both total and IgG4 anti-PLA2R1 activity using a sensitive and quantitative ELISA.

Despite the relatively small number of patients of our study, we had at least one case to re-evaluate the previously reported discrepancies between anti-PLA2R1 activity and MN

recurrence [22]. First, patients with anti-PLA2R1 antibody at the time of renal transplantation may not present MN recurrence because of a rapid decrease of anti-PLA2R1 activity before Month 6 following transplant immunosuppression. Second, proteinuria may be masked by combined RAS blockade and high doses of CNI as shown previously [5]. Therefore, persistently high anti-PLA2R1 antibody titres with no proteinuria should lead to suspect sub-clinical recurrent MN. In this regard, we cannot rule out a higher rate of sub-clinical MN recurrence in our patients, as renal biopsies were not always performed in the absence of overt proteinuria. Third, MN recurrence with apparently no anti-PLA2R1 activity may be due to the lower sensitivity of total IgG assays that were used in previous studies including IIFT [22]. Indeed, we found that measuring IgG4 anti-PLA2R1 activity by ELISA is more sensitive than measuring total IgG, either by ELISA or by IIFT (see Table 1 and Materials and Methods). Importantly, the monitoring of IgG4 anti-PLA2R1 activity was better associated with MN disease activity than total IgG [5]. Fourth, the increase of proteinuria in a patient who exhibited a total disappearance of anti-PLA2R1 antibodies suggests a differential diagnosis. Fifth, in patients without anti-PLA2R1 antibodies, MN may also recur after renal transplantation, suggesting in this case the presence of an unknown antigen target.

Overall, we observed that although the titre of anti-PLA2R1 antibody at the time of renal transplantation was not predictive of MN recurrence ($P = 0.600$, $n = 15$), a persistently positive anti-PLA2R1 activity after 6 months of follow-up was significantly associated with MN recurrence ($P = 0.0048$, $n = 10$). Interestingly, this persistent activity was only observed in patients who did not receive the strongest immunosuppressive regimen with both induction therapy and combined treatment with CNI and mycophenolate. These results seem to contradict the study of Mulay *et al.* [9] that measured the impact of maintaining immunosuppressive treatments on the rate of recurrence of various primary glomerulonephritides. However, Mulay *et al.* did not analyse the impact of induction therapies and studied a large number of different types of glomerulonephritides including MN but without measuring anti-PLA2R1 activity.

We conclude from our study that patients with anti-PLA2R1 activity at the time of renal transplantation should benefit, in addition to the measurements of biological parameters such as proteinuria and serum creatinine, from the subsequent monitoring of this auto-reactivity (especially IgG4) by a sensitive ELISA to predict MN recurrence and distinguish MN from differential diagnoses. Furthermore, baseline anti-PLA2R1 antibody titres, immunosuppressive regimen and persistent IgG4 anti-PLA2R1 activity after Month 6 would likely help to predict MN recurrence. Our suggestion that both IgG4 anti-PLA2R1 antibody titres and mild immunosuppressive regimens may be involved in MN recurrence should now be confirmed in an ongoing large prospective French collaborative study (NCT01897961).

SUPPLEMENTARY DATA

Supplementary data are available online at <http://ndt.oxfordjournals.org>.

AUTHORS' CONTRIBUTIONS

B.S.P., G.L. and V.L.M.E. participated in the research design, in the analysis of the data and in drafting and revising the article. B.S.P. and C.P. performed the anti-PLA2R1-specific assays under the supervision of S.B., G.B. and G.L. B.S.P., E.C., M.B., L.A., M.C.T. and A.J. collected the clinical data. D.A. performed the pathological studies. All authors revised and gave final approval of the last version of this article.

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CONFLICT OF INTEREST STATEMENT

None declared.

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