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A New Chemiluminescence Immunoassay for Phospholipase A_2 Receptor 1 Autoantibodies Allows Early Identification of Autoantibody Recurrence in Patients With Membranous Nephropathy

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Background: Circulating autoantibodies against the M-type phospholipase A_2 receptor 1 (PLA_2R1) are important biomarkers in membranous nephropathy (MN), supporting the diagnosis and the clinical monitoring of patients. Standardized recombinant cell-based indirect immunofluorescence assay (RC-IFA) and enzyme-linked immunosorbent assay (ELISA) are widely established for the detection of anti-PLA_2R1 autoantibodies (PLA_2R1-ab). The RC-IFA provides higher sensitivity than the ELISA, but lacks exact graduated quantification of antibody levels. In this study, we evaluated the diagnostic performance of a novel PLA_2R1-ab immunoassay based on chemiluminescence (ChLIA) by comparing it to RC-IFA and ELISA in samples from patients with MN with different diagnostic scenarios.

Methods: Serum samples from patients with biopsy-proven MN and disease controls were analyzed for PLA_2R1-ab by ChLIA, ELISA, and RC-IFA.

Results: The ChLIA demonstrated almost perfect agreement with RC-IFA for the identification of patients with PLA_2R1-associated MN, while additionally allowing fine-graduated quantification of PLA_2R1-ab levels. In patients with a relapse of MN, the ChLIA allowed an earlier detection of PLA_2R1-ab recurrence by at least 3 months in 63% of cases compared with the ELISA.

Conclusions: The PLA_2R1-ab ChLIA had the same excellent diagnostic performance as the RC-IFA and outperformed the ELISA in the diagnosis of MN and the early identification of relapses. It thus presents a favorable tool for accurate PLA_2R1-ab assessment in routine diagnostic settings, while enabling fast processing and fully automated random-access implementation.

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MN is the most common cause of a nephrotic syndrome in Caucasian adults.[1,2] The worldwide annual incidence is approximately 12 per million and affects men twice as often as women, with a predominance of onset in the older individuals (mean age 50–60 years).[1,2] MN is characterized by subepithelial deposition of immune complexes and complement components, leading to changes in the glomerular filtration barrier, which results in proteinuria and renal failure in up to one-third of cases.[3,4]

PLA_2R1 has been known for the past decade as the major target antigen of circulating and glomerulideposited PLA_2R1-ab, which is found in approximately 70% of adult patients with MN and predominantly belongs to the IgG4 subclass.[5–7] Furthermore, the podocyte membrane antigen thrombospondin type-1 domain-containing protein 7A was identified as the second target of autoantibodies in 2% to 3% of MN cases.[8–11] Dual positivity for PLA_2R1-ab and thrombospondin type-1 domain-containing protein 7A-ab has been reported for only a few cases.[9] The discovery of the major target antigen has quickly

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been translated into clinical practice: PLA_2R1-ab has become a valuable biomarker for the diagnosis of MN, and sequential PLA_2R1-ab titers are used for monitoring the disease activity, assessment of the therapy response, and for prognostic evaluation (i.e., prediction of therapy outcome, relapses or spontaneous remission and long-term renal outcome), as reported and reviewed elsewhere.[3,12–15]

Today, the standardized detection of autoantibodies against PLA_2R1 is possible by means of various assays.[3,12] RC-IFA (EUROIMMUN Medizinische Labordiagnostika AG, Lübeck, Germany) is a highly sensitive method, with particular capability in detecting very low PLA_2R1-ab titers.[7,16,17] Devices enabling fully automated incubation, microscopy, and evaluation of RC-IFA are available. However, because of the lack of fine-graduated quantification, it is less useful for patient monitoring. For a more precise determination of antibody concentrations, a standardized ELISA (EUROIMMUN) and an addressable laser bead immunoassay (Mitogen Advanced Diagnostics Laboratory, Calgary, Alberta, Canada) were developed, enabling quantitative evaluation and robust automated testing in high-throughput routine settings.[17–20]

The ELISA is widely used for diagnosis and follow-up of MN. It has been shown to provide a very high specificity and a high correlation with RC-IFA; however, the sensitivity of the ELISA is slightly lower, which is particularly relevant when testing sera from patients during disease remission.[17,21] This resulted in a controversy as to whether the diagnostic cutoff of the ELISA should be lowered. Consequently, when the cutoff was lowered, the specificity decreased from 100% to 96.8%.[19,20,22–25]

Of note, PLA_2R1-ab titers correlate well with the disease course. In this regard, fine-graduated quantification of the antibody levels is crucial, which is provided by ELISA but not by RC-IFA.[16,26,27]

In the study presented here, we aimed to evaluate the performance of a novel standardized ChLIA[28] for the detection of PLA_2R1-ab in samples from patients with MN at different disease stages, especially in clinically relevant follow-up scenarios, and to investigate whether the ChLIA can close the sensitivity gap between ELISA and RC-IFA.

MATERIALS AND METHODS

Patients and Samples

The study included serum samples from 327 patients with MN from a prospective cohort. Clinical characteristics of 280 of these patients have been published earlier.[11,15] MN was diagnosed by renal biopsy in all 327 patients. The PLA_2R1-ab status was determined by ELISA, RC-IFA, and Western blot. Patients were divided in the following cohorts, depending on their PLA_2R1-ab status (Figure 1):

- Cohort A contained 262 sera from patients with PLA_2R1-ab–positive MN. The first serum collection for PLA_2R1-ab measurement was performed within 6 months of kidney biopsy in all patients.
- Cohort B contained 65 sera from patients with PLA_2R1-ab–negative MN. The first serum collection for PLA_2R1-ab measurement was performed within 6 months of kidney biopsy in 60 of 65 patients.
- As a control cohort C, 60 sera from patients with a biopsy-proven diagnosis of glomerulonephritis, excluding MN, were included in the analyses.

Follow-up sera were collected at every patient visit, which took place at intervals of 3 to 6 months. We analyzed 2 series of follow-up samples from a subset of cohort A patients who displayed the following clinical settings relevant for treatment management of MN:

- Cohort A1: 45 patients with 1 or 2 recurrences of PLA_2R1-ab positivity during follow-up.

In these patients, PLA_2R1-ab became undetectable by ELISA, but recurred during follow-up. A total of 52 incidents of immunological relapses were documented in this cohort, comprising a total of 221 sera collected at the time when PLA_2R1-ab became detectable again by ELISA (relapsed) and at the last 2 visits before PLA_2R1-ab recurrence. If all 3 sequential sera were positive for PLA_2R-ab by ChLIA, more follow-up samples collected before the PLA_2R1-ab recurrence were analyzed.

- Cohort A2: 15 patients, in whom PLA_2R1-ab became negative but proteinuria persisted or relapsed.

This cohort included 44 sera that were determined as negative for PLA_2R1-ab by ELISA. All patients had been PLA_2R1-ab positive at study inclusion and were part of cohort A. At the time of collection, 2 patients had no remission of proteinuria, whereas the remaining 13 patients experienced a relapse of proteinuria after partial or complete remission (8 and 5 patients, respectively).

All sera were stored at −20°C. For serological analysis, all clinical data were anonymized and blinded. The study was approved by the local ethics committee of the chamber of physicians in Hamburg, Germany, and conducted in accordance with the ethical principles stated by the Declaration of Helsinki. An informed consent was obtained from all participating patients.

Immunoassays

Determination of PLA_2R1-ab was performed with commercially available ChLIA, ELISA, and RC-IFA test

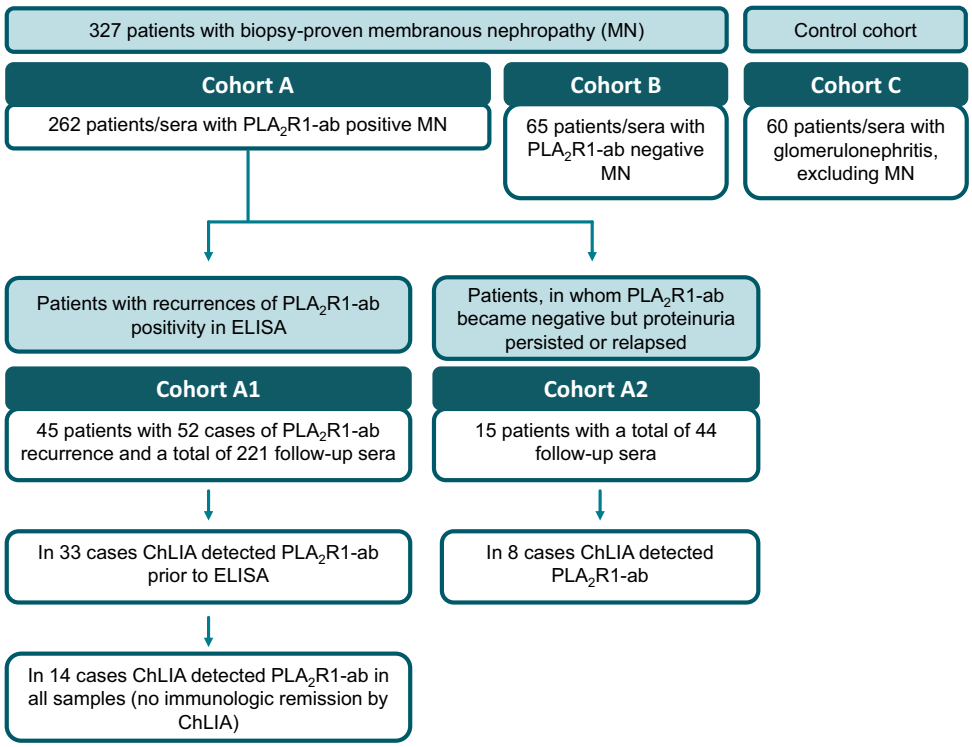

Figure 1. Patient cohorts and their PLA_2R1-ab status.
ChLIA, chemiluminescence immunoassay; ELISA, enzyme-linked immunosorbent assay; MN, membranous nephropathy; PLA_2R1-ab, phospholipase A_2 receptor 1-antibody.

kits (EUROIMMUN), as described previously.[28] As recommended by the manufacturer, PLA_2R1-ab results in the ELISA were considered positive at a level ≥20 relative units (RU)/ml and borderline when ≥14 to <20 RU/ml. For thrombospondin type-1 domain-containing protein 7A-ab detection, the RC-IFA test kit (EUROIMMUN) was used, as described previously [11].

Statistics

The data were evaluated statistically using GraphPad Prism 6, GraphPad Prism QuickCalcs (GraphPad Software Inc., La Jolla, CA) and SigmaPlot 13.0 (SSI, San Jose, CA). Kappa statistic (κ) and its 95% confidence interval were used to assess the level of agreement of PLA_2R1-ab detection between ChLIA and RC-IFA or ELISA.[29]

RESULTS

The prospective sample collection of most patients with biopsy-proven MN (Table 1) was described previously.[11,15] For the purpose of retrospectively evaluating the performance of the novel PLA_2R1-ab chemiluminescence assay, samples from this collection were analyzed with all commercially available test systems: ChLIA, ELISA, and RC-IFA.

First, sera collected at the time of study inclusion were tested, representing the time of MN diagnosis (Table 2). ChLIA demonstrated the highest percentage of positive agreement (95.0%) with PLA_2R1-ab positivity, classified by Western blot, and performed

Table 1. Baseline characteristics of PLA_2R1-ab positive (cohort A) and PLA_2R1-ab negative (cohort B) patients included in the study

Cohort	A: MN – PLA_2R1-ab positive (n = 262)	B: MN – PLA_2R1-ab negative (n = 65)
Age, y (median, 1st–3rd quartile)	56.0, 43.3–67.0	60.0, 48.0–70.0
Gender, male (%)	187 (71.4)	36 (55.4)
Serum creatinine, mg/dl (median, 1st–3rd quartile)	1.0, 0.8–1.3	1.0, 0.7–1.4
eGFR, CKD-EPI, ml/min per 1.73 m^2 (median, 1st–3rd quartile)	56.0, 43.3–67.0	60.0, 48.0–70.0
Proteinuria (g/d)	6.7, 4.4–10.1	4.1, 2.2–6.7
PLA_2R1 staining positive in the kidney biopsy (%, n = 142 for cohort A and n = 26 for cohort B)	140 (98.6)	1 (3.8)
% tubular atrophy and interstitial fibrosis (median, 1st to 3rd quartile)	10.0, 0.0–20.0 (n = 247)	5.0, 0.0–16.3 (n = 49)
Glomerular lesions in renal biopsies (electron microscopy, n = 243 for cohort A and n = 50 for cohort B)		
Stage 1 (%)	17 (7.0)	8 (16.0)
Stage 2 (%)	139 (57.2)	24 (48.0)
Stage 3 (%)	46 (18.9)	11 (22.0)
Stage 4 (%)	41 (16.9)	7 (14.0)
Time from kidney biopsy to first serum collection, months (median, 1st–3rd quartile)	0.8, 0.3–1.0	0.8, 0.3–2.0
Immunosuppressive treatment during follow-up (%)	183 (70.4)	20 (30.8)

CKD-EPI, Chronic Kidney Disease–Epidemiology Collaboration; eGFR: estimated glomerular filtration rate; MN, membranous nephropathy; PLA_2R1-ab, phospholipase A_2 receptor 1-antibody.

Table 2. Comparison of the PLA_2R1-ab ChLIA, ELISA, and RC-IFA according to the applied patient cohort as categorized based on previous Western blot data and biopsy

		PLA_2R1-ab positive		
Cohort	*n*	PLA_2R1-ab ChLIA (cutoff 10 CU/ml)[a]	PLA_2R1-ab ELISA (cutoff 20 RU/ml)[a,b]	PLA_2R1-ab RC-IFA (Cutoff titer 1:10)[a]
A (MN – PLA_2R-ab positive)	262	249	230	248
Positive agreement,[c] %		95.0	87.8	94.7
B (MN – PLA_2R-ab negative)	65	2	0	0
C (GN – excluding MN)	60	0	0	0
Negative agreement,[c] %		98.4	100	100

ChLIA, chemiluminescence immunoassay; CU/ml, chemiluminescent units per milliliter; ELISA, enzyme-linked immunosorbent assay; GN, glomerulonephritis; MN, membranous nephropathy; PLA_2R1-ab, phospholipase A_2 receptor 1-antibody; RC-IFA, recombinant cell-based indirect immunofluorescence assay; RU, relative units.
[a]Cutoff recommended by the manufacturer.
[b]Borderline results (≥14 to <20 RU/ml) were considered as negative.
[c]Compared with Western blot data.

similar to RC-IFA (94.7%). The ELISA showed lowest percentage of positive agreement (87.8%) of all methods. Testing samples from PLA_2R1-ab–negative patients resulted in 2 positive findings with ChLIA. For one of these patients, kidney biopsy tissue was available for PLA_2R1 staining, which resulted negative, confirming that this patient did not have PLA_2R1-associated MN.

Assessment of agreement between methods revealed the highest agreement for RC-IFA and ChLIA ($\kappa = 0.98$; 95% confidence interval: 0.964–1.002). The agreement between ChLIA and ELISA was slightly lower, but also almost perfect ($\kappa = 0.89$; 95% confidence interval 0.837–0.933). The PLA_2R1-ab level measured by ELISA and ChLIA showed a strong correlation (rho = 0.91; $P < 0.0001$).

To evaluate the testing performance for PLA_2R1-ab monitoring in clinical management, longitudinal follow-up samples from patients with a recurrence of PLA_2R1-ab in ELISA (patient cohort A1) were examined. At the time when the 52 patient cases were determined to have reoccurring PLA_2R1-ab titer by ELISA, all were found to be PLA_2R1-ab positive by ChLIA, as well (Figure 2). Interestingly, in 33 of 52 cases (63%) PLA_2R1-ab was detected by ChLIA in the samples collected at the prior study visit, which was performed in the median 3 months before the ELISA yielded a positive result. Eleven of the 52 samples (21%) showed a borderline PLA_2R1-ab level by ELISA at this time point. Furthermore, for 44 of the 52 cases, a second, earlier serum sample was available, which was collected in the median 6 months before the ELISA yielded a positive result for PLA_2R1-ab. Sixteen of these 44 samples (36%) were PLA_2R1-ab positive by ChLIA. Four sera had a borderline PLA_2R1-ab level measured by ELISA.

For those patients, who resulted PLA_2R1-ab positive by ChLIA in all 3 consecutive measurements and had further follow-up samples available, the comparison was extended. Taken together, in cohort A1, we identified 14 cases of ELISA-defined immunological remission, followed by a relapse that were positive for PLA_2R1-ab by ChLIA throughout all the tested samples and thus did neither have an antibody remission nor a relapse as defined by ELISA. Six of these cases showed a borderline PLA_2R1-ab level measured by ELISA. Excluding the 14 cases that were positive for PLA_2R1-ab by ChLIA throughout all tested samples, ChLIA detected a PLA_2R1-ab recurrence in the median 6 months ahead of the ELISA in half of the cases (19 of 38) with immunological relapse confirmed by both methods.

The clinical data from patients grouped in cohort A1 were analyzed for their disease activity, regarding the progression of proteinuria. In 42 of the 52 examined follow-up cases, patients had a remission of proteinuria, which deteriorated into a relapse of nephrotic proteinuria ($n = 26$) or a development of proteinuria of 0.5 to 3.5 g/d (formally partial remission) after the patients had a complete remission of proteinuria (defined as proteinuria <0.5 g/d) ($n = 16$). Five cases presented remission of proteinuria without documented deterioration despite the reoccurrence of PLA_2R1-ab positivity, although in 1 case no follow-up after relapse of PLA_2R1-ab was available. The remaining 5 cases had not entered remission of proteinuria during the time in which PLA_2R1-ab had temporarily become negative.

Considering only the 33 cases, in which PLA_2R1-ab were detectable by ChLIA at a time point earlier than by ELISA, in 18 patients immunologic relapse was accompanied by a relapse of proteinuria. Further, 8 patients developed proteinuria of 0.5 to 3.5 g/d (partial remission) after they had a complete remission, 4 patients had no remission of proteinuria during the time when PLA_2R1-ab was negative, 2 patients had a persistent partial remission of proteinuria, and for 1 patient no follow-up data were available. Interestingly, among the 26 patients with relapse of proteinuria and early detection of PLA_2R1-ab recurrence by ChLIA, the median time from immunological relapse of PLA_2R1-ab by ELISA until relapse of proteinuria was 0.5 months. In comparison, among the 16 patients with relapse of proteinuria and no early detection of PLA_2R1-ab recurrence by ChLIA (patients, in whom detection of the PLA_2R1-ab relapse by ELISA and ChLIA took place at the same time), the median time from immunological relapse until relapse of proteinuria was 3 months. This

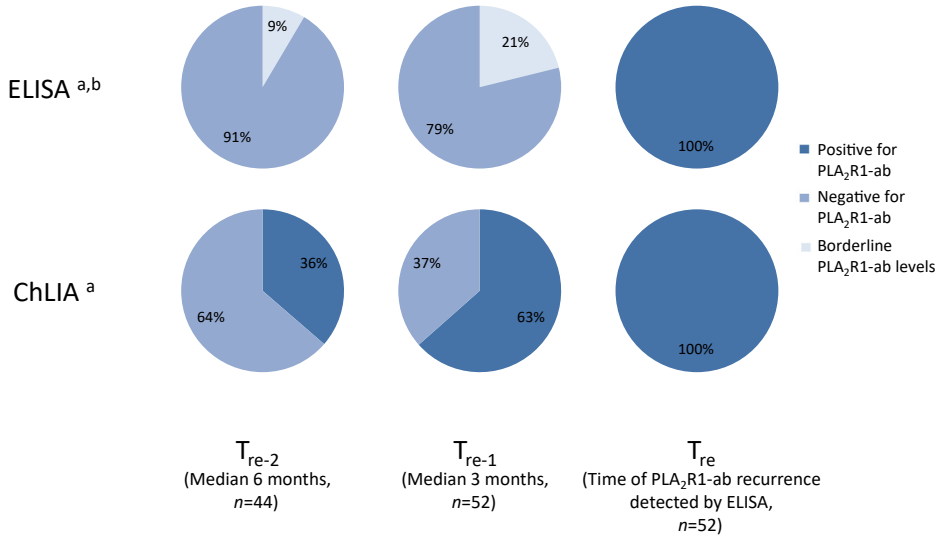

Figure 2. Temporal comparison of PLA_2R1-ab positivity by ELISA and ChLIA. PLA_2R1-ab positivity determined by ChLIA method is compared with reference to PLA_2R1-ab recurrence detected by ELISA at the time point T_{re} for every case (52 cases in total from cohort A1). On the left, the comparison between both methods in sera collected at a median of 6 months before T_{re} shows that ChLIA already detected 36% positive samples. The detection rate of ChLIA increased to 63% positive samples in sera collected at a median time of 3 months before T_{re} (middle section). At both times before T_{re}, ELISA showed no PLA_2R1-ab positivity; however, 9% and 21% of sera, respectively, showed borderline PLA_2R1-ab levels in the ELISA. The right hand side shows 100% PLA_2R1-ab positivity at T_{re} detected by both methods. ChLIA, chemiluminescence immunoassay; ELISA, enzyme-linked immunosorbent assay; PLA_2R1-ab, phospholipase A_2 receptor 1-antibody.
[a]Cutoff recommended by the manufacturer (ELISA 20 RU/ml, ChLIA 10 CU/ml).
[b]Borderline PLA_2R1-ab levels were defined as $\geq$14 to $<$20 RU/ml.

suggests that the "early" PLA_2R1-ab reappearance by ChLIA is indeed associated with an earlier relapse of proteinuria.

In total, 49 analyzed follow-up sera showed a positive PLA_2R1-ab detection by ChLIA and a negative PLA_2R1-ab report by ELISA. All these sera were analyzed by RC-IFA and showed a positive PLA_2R1-ab result in 43 cases (88%).

As the ChLIA thus far demonstrated enhanced detection rates of PLA_2R1-ab at a comparable level of RC-IFA, the performance of both tests was evaluated in 15 patients (cohort A2) in whom PLA_2R1-ab had become negative and was continuously determined as negative by ELISA, despite proteinuria persistence or relapse. In this cohort, we aimed to investigate whether the discrepancy between the immunological (PLA_2R1-ab) and clinical (proteinuria) disease activity was caused by persistent glomerular damage or insufficient sensitivity of PLA_2R1-ab detection. Measurements with ChLIA and RC-IFA concordantly revealed the presence of PLA_2R1-ab above cutoff levels in 8 of the 15 patients (1 with persistent proteinuria, 5 and 2 with relapse of proteinuria after partial or complete remission, respectively). Two of these patients showed borderline PLA_2R1-ab levels by ELISA. One patient, who had a relapse of proteinuria, was positive for PLA_2R1-ab in the RC-IFA, but negative by ChLIA and ELISA. The remaining 6 patients showed persistent or relapsing proteinuria but were negative for PLA_2R1-ab by ChLIA, RC-IFA, and ELISA. PLA_2R1 staining in the kidney biopsy was available for 5 of these patients and was positive in all cases. A concurrent glomerular disease was not diagnosed in any of the biopsies. Tubular atrophy and interstitial fibrosis was severe in 2 biopsies (45% and 50%, respectively) and mild in the remaining cases (0%–5%), whereas glomerular lesions (by electron microscopy) were classified as class 2 in 4 cases and class 3 in 2 cases. Moreover, in 2 biopsies, hypertensive damage was found, in 1 case mild and 1 case moderate. The sera of all 6 patients were tested for thrombospondin type-1 domain-containing protein 7A-ab and all resulted negative.

DISCUSSION

Although kidney biopsy remains the diagnostic gold standard, PLA_2R1-ab testing is a noninvasive tool for diagnosing MN, especially in patients with a high risk of complications and contraindication to biopsy.[3,25,30]

In the present study, we investigated the role of a novel PLA_2R1-ab ChLIA for the monitoring of patients with MN, comparing the analytical results of ChLIA with the established ELISA and RC-IFA in serum samples from a previous prospective study.[11,15] In the analysis of samples representing the initial diagnosis of MN, the ChLIA demonstrated a detection rate that was concordant to the RC-IFA and considerably increased compared with the ELISA. This is consistent with

another report about the analytical performance of the ChLIA.[28] Moreover, in the study presented here, a similar outcome was found in the analysis of longitudinal follow-up samples. The ChLIA revealed a recurrence of PLA_2R1-ab positivity earlier than the ELISA. Besides, it identified persistent PLA_2R1-ab levels that were otherwise considered as negative by ELISA.

Thus far, the ELISA presented the only worldwide available tool for fine-graduated quantification of PLA_2R1-ab levels in patient monitoring. Due to its lower sensitivity compared with RC-IFA, Bobart *et al.*[25] recommended a confirmation by RC-IFA in case of samples showing ELISA values in the range between $\geq$2 and $\leq$20 RU/ml. Our data indicate that the novel ChLIA is capable of closing the reported sensitivity gap between ELISA and RC-IFA.[17] Moreover, the sensitivity of ChLIA to detect PLA_2R1-ab is higher than the ELISA, even when "borderline" PLA_2R1-ab levels in the ELISA are considered positive. The overall diagnostic performance of the ChLIA was nearly identical with that of RC-IFA. In the cohort addressing the initial diagnosis, only 2 samples were discrepant and exclusively PLA_2R1-ab reactive by ChLIA. In 1 of the cases for which kidney tissue was available, PLA_2R1 staining of the biopsy did not show PLA_2R1 positivity.

Several groups demonstrated a decrease or disappearance of PLA_2R1-ab levels on treatment or spontaneous remission,[12] the latter occurring in approximately one-third (32%) of patients with MN.[31] However, relapses also occur. Hitherto, the RC-IFA represents the most precise method because of its higher sensitivity for anticipating relapses based on the reoccurrence of PLA_2R1-ab. Here, we demonstrated that the ChLIA likewise provides an excellent tool, detecting PLA_2R1-ab substantially earlier compared with ELISA. It became clear in this study that the determination of PLA_2R1-ab is important not just for the diagnosis but also for the monitoring of patients. Further prospective studies should address the question of whether the improvement in the PLA_2R1-ab detection and titer measurement by ChLIA will lead to an improvement of the disease outcome, that is, prevention of renal insufficiency or end-stage kidney disease. However, the data presented here already reveal that patients with persistent proteinuria but temporal absence of PLA_2R1-ab positivity in ELISA often have persistent PLA_2R1-ab by ChLIA. Thus, the ChLIA has the potential to indicate low levels of circulating PLA_2R1-ab undetected by ELISA, which could be causative for proteinuria and might require immunosuppressive therapy for the patient.

At the same time, it should be considered that PLA_2R1-ab may occur months or even years before the clinical presentation and diagnosis of MN.[32] Therefore, it is possible that for the onset of clinical symptoms, PLA_2R1-ab may have to exceed certain thresholds concerning both PLA_2R1-ab level and PLA_2R1-ab persistence time in the blood. In some cases, PLA_2R1-ab may also rapidly disappear without need of immunosuppressive treatment, as seen in patients with low PLA_2R1-ab levels who develop a spontaneous remission of disease. Therefore, follow-up measurement of PLA_2R1-ab is very important in the clinical management of these patients.

Notably, in this study, the enhanced detection rate of the ChLIA required a revision in the analysis of the data comprising patients with recurring PLA_2R1-ab positivity after immunological remission. In 14 cases, all available samples were found positive by ChLIA and, therefore, would have been falsely classified as "remission" of PLA_2R1-ab in routine ELISA testing. In some of these patients, however, proteinuria developed concordantly to PLA_2R1-ab, meaning that a remission of proteinuria occurred, followed by a relapse, showing that the PLA_2R1-ab level was clinically important. We also analyzed patients who showed discordant findings of PLA_2R1-ab and proteinuria. These patients had persistent or relapse of proteinuria, although PLA_2R1-ab levels were negative in ELISA. In these cases, proteinuria might result from glomerular damage either by PLA_2R1-ab entrapped in the glomeruli or due to another disease (i.e., diabetic nephropathy), both of which are not addressable by immunosuppressive therapy. On the other hand, low levels of circulating PLA_2R1-ab, which might remain undetected by ELISA, also could be causative of proteinuria, and require a more aggressive immunosuppressive treatment. We identified PLA_2R1-ab in more than 50% of patients in cohort A2, who were characterized as PLA_2R1-ab negative by ELISA but had persisting or relapsing proteinuria. Moreover, 4 of the 5 patients in cohort A1, who did not go into remission of proteinuria but showed a remission of PLA_2R1-ab in ELISA, showed constant PLA_2R1-ab positivity in ChLIA. The possible clinical benefits of the transfer of these findings into routine application need to be addressed prospectively in future studies. The routine application of new PLA_2R1-ab measurement techniques such as the ChLIA described in this article, or addressable laser bead immunoassay, should be confirmed in future studies.

In summary, the diagnostic performance of the ChLIA is comparable to that of the RC-IFA, but exceeds that of the ELISA. A further advantage of the ChLIA is the possibility of random-access full automation and fine-graduated quantification. Promising to overcome the previously reported sensitivity gap between the established ELISA and RC-IFA, the ChLIA is

advantageous in routine application, especially in patient monitoring.

DISCLOSURE

EUROIMMUN Medizinische Labordiagnostika AG is the exclusive licensee of patents pertaining to the detection of autoantibodies to PLA_2R1. AK and CD are employees of EUROIMMUN, a company that develops and manufactures immunoassays for the detection of disease-associated antibodies. WS is a board member of EUROIMMUN. All the other authors declared no competing interests. Company staff were involved in data analysis and writing of the manuscript. However, the contributions of EUROIMMUN employees were based exclusively on scientific grounds and transparent to and reviewable by all coauthors. The corresponding author had full access to all study data and had final responsibility for the decision to submit for publication.

The results presented in this paper have not been published previously in whole or part, except in abstract form.

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REFERENCES

1. McGrogan A, Franssen CF, de Vries CS. The incidence of primary glomerulonephritis worldwide:a systematic review of the literature. *Nephrol Dial Transplant*. 2011;26:414–430.
2. Couser WG. Primary membranous nephropathy. *Clin J Am Soc Nephrol*. 2017;12:983–997.
3. De Vriese AS, Glassock RJ, Nath KA, et al. A proposal for a serology-based approach to membranous nephropathy. *J Am Soc Nephrol*. 2017;28:421–430.
4. Debiec H, Ronco P. Immunopathogenesis of membranous nephropathy:an update. *Semin Immunopathol*. 2014;36:381–397.
5. Beck LH Jr, Bonegio RG, Lambeau G, et al. M-type phospholipase A2 receptor as target antigen in idiopathic membranous nephropathy. *N Engl J Med*. 2009;361:11–21.
6. Qin W, Beck LH Jr, Zeng C, et al. Anti-phospholipase A2 receptor antibody in membranous nephropathy. *J Am Soc Nephrol*. 2011;22:1137–1143.
7. Hofstra JM, Debiec H, Short CD, et al. Antiphospholipase A2 receptor antibody titer and subclass in idiopathic membranous nephropathy. *J Am Soc Nephrol*. 2012;23:1735–1743.
8. Tomas NM, Beck LH Jr, Meyer-Schwesinger C, et al. Thrombospondin type-1 domain-containing 7A in idiopathic membranous nephropathy. *N Engl J Med*. 2014;371:2277–2287.
9. Larsen CP, Cossey LN, Beck LH. THSD7A staining of membranous glomerulopathy in clinical practice reveals cases with dual autoantibody positivity. *Mod Pathol*. 2016;29:421–426.
10. Tomas NM, Hoxha E, Reinicke AT, et al. Autoantibodies against thrombospondin type 1 domain-containing 7A induce membranous nephropathy. *J Clin Invest*. 2016;126:2519–2532.
11. Hoxha E, Beck LH Jr, 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;28:520–531.
12. Schlumberger W, Hornig N, Lange S, et al. Differential diagnosis of membranous nephropathy with autoantibodies to phospholipase A2 receptor 1. *Autoimmun Rev*. 2014;13:108–113.
13. Ronco P, Debiec H. Pathophysiological advances in membranous nephropathy:time for a shift in patient's care. *Lancet*. 2015;385:1983–1992.
14. Radice A, Pieruzzi F, Trezzi B, et al. Diagnostic specificity of autoantibodies to M-type phospholipase A2 receptor (PLA2R) in differentiating idiopathic membranous nephropathy (IMN) from secondary forms and other glomerular diseases. *J Nephrol*. 2018;31:271–278.
15. Mahmud M, Pinnschmidt HO, Reinhard L, et al. Role of phospholipase A2 receptor 1 antibody level at diagnosis for long-term renal outcome in membranous nephropathy. *PLoS One*. 2019;14:e0221293.
16. Hoxha E, Harendza S, Zahner G, et al. An immunofluorescence test for phospholipase-A_2-receptor antibodies and its clinical usefulness in patients with membranous glomerulonephritis. *Nephrol Dial Transplant*. 2011;26:2526–2532.
17. Dähnrich C, Komorowski L, Probst C, et al. Development of a standardized ELISA for the determination of autoantibodies against human M-type phospholipase A2 receptor in primary membranous nephropathy. *Clin Chim Acta*. 2013;421:213–218.
18. Behnert A, Fritzler MJ, Teng B, et al. An anti-phospholipase A2 receptor quantitative immunoassay and epitope analysis in membranous nephropathy reveals different antigenic domains of the receptor. *PLoS One*. 2013;8:e61669.
19. Behnert A, Schiffer M, Müller-Deile J, et al. Antiphospholipase A_2 receptor autoantibodies: a comparison of three different immunoassays for the diagnosis of idiopathic membranous nephropathy. *J Immunol Res*. 2014;2014:143274.
20. Timmermans SA, Damoiseaux JG, Heerings-Rewinkel PT, et al. Evaluation of anti-PLA2R1 as measured by a novel ELISA in patients with idiopathic membranous nephropathy: a cohort study. *Am J Clin Pathol*. 2014;142:29–34.
21. Li X, Wei D, Zhou Z, et al. Anti-PLA2R antibodies in Chinese patients with membranous nephropathy. *Med Sci Monit*. 2016;22:1630–1636.
22. Dou Y, Zhang L, Liu D, et al. The accuracy of the anti-phospholipase A2 receptor antibody in the diagnosis of idiopathic membranous nephropathy:a comparison of different cutoff values as measured by the ELISA method. *Int Urol Nephrol*. 2016;48:845–849.

23. Liu Y, Li X, Ma C, et al. Serum anti-PLA2R antibody as a diagnostic biomarker of idiopathic membranous nephropathy: the optimal cut-off value for Chinese patients. *Clin Chim Acta*. 2018;476:9–14.
24. Tampoia M, Migliucci F, Villani C, et al. Definition of a new cut-off for the anti-phospholipase A2 receptor (PLA2R) autoantibody immunoassay in patients affected by idiopathic membranous nephropathy. *J Nephrol*. 2018;31:899–905.
25. Bobart SA, De Vriese AS, Pawar AS, et al. Noninvasive diagnosis of primary membranous nephropathy using phospholipase A2 receptor antibodies. *Kidney Int*. 2019;95:429–438.
26. Beck LH Jr, Fervenza FC, Beck DM, et al. Rituximab-induced depletion of anti-PLA2R autoantibodies predicts response in membranous nephropathy. *J Am Soc Nephrol*. 2011;22:1543–1550.
27. Hoxha E, Thiele I, Zahner G, et al. Phospholipase A2 receptor autoantibodies and clinical outcome in patients with primary membranous nephropathy. *J Am Soc Nephrol*. 2014;25:1357–1366.
28. Dähnrich C, Saschenbrecker S, Gunnarsson I, et al. Development of a standardized chemiluminescence immunoassay for the detection of autoantibodies against human M-type phospholipase A2 receptor in primary membranous nephropathy. *Kidney Int Rep*. 2020;5:182–188.
29. Viera AJ, Garrett JM. Understanding interobserver agreement: the kappa statistic. *Fam Med*. 2005;37:360–363.
30. Wiech T, Stahl RAK, Hoxha E. Diagnostic role of renal biopsy in PLA(2)R1-antibody-positive patients with nephrotic syndrome. *Mod Pathol*. 2019;32:1320–1328.
31. Polanco N, Gutiérrez E, Covarsí A, et al. Spontaneous remission of nephrotic syndrome in idiopathic membranous nephropathy. *J Am Soc Nephrol*. 2010;21:697–704.
32. Burbelo PD, Joshi M, Chaturvedi A, et al. Detection of PLA2R autoantibodies before the diagnosis of membranous nephropathy. *J Am Soc Nephrol*. 2020;31:208–217.