J Nephrol (2014) 27:111–116
DOI 10.1007/s40620-014-0042-7

REVIEW

Diagnostic value of phospholipase A_2 receptor in idiopathic membranous nephropathy: a systematic review and meta-analysis

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Received: 31 August 2013 / Accepted: 21 October 2013 / Published online: 6 February 2014

Abstract
Background Detection of M-type phospholipase A_2 receptor (PLA_2R) can be used in serologic diagnosis of idiopathic membranous nephropathy (IMN), but there are limited data about the sensitivity and specificity of its diagnostic values.
Methods and results Meta-analysis of diagnostic test studies assessing the values of PLA_2R in diagnosis of IMN. MEDLINE, EMBASE, and CENTRAL databases and congress abstracts were searched for studies reporting the value of PLA_2R to predict IMN. The quality of the studies was evaluated using the guidelines of the updated Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) tool. The results are summarized as sensitivity, specificity, and diagnostic odds ratio (OR). Data from 10 studies involving 1,550 participants were analyzed. Across all settings, the diagnostic OR for serum anti-PLA_2R level to predict IMN at different stages was 247.41, with sensitivity of 0.69 and specificity of 0.99. The estimated sensitivity and specificity of serum anti-PLA_2R level for diagnosis of IMN in the active stage were 74.0 and 95.0 %, respectively, with diagnostic OR of 54.22. The estimated sensitivity and specificity of biopsy anti-PLA_2R for diagnosis of IMN at different stages was 73.0 and 83.0 %, respectively, with diagnostic OR of 13.75.
Conclusions This meta-analysis shows that serum anti-PLA_2R level is of diagnostic value for IMN in the active stage. Future large-cohort prospective studies are required to reveal the diagnostic value of circulating anti-PLA_2R antibodies versus PLA_2R antigens in kidney biopsy for IMN at different stages.

Keywords Anti-phospholipase A_2 receptor · Idiopathic membranous nephropathy · Diagnostic value · Systematic review · Meta-analysis

Introduction

Idiopathic membranous nephropathy (IMN) remains the major cause of adult nephrotic syndrome, with up to one-third of patients progressing to end stage renal disease (ESRD) [1, 2]. IMN has long been suspected of having an immune etiology, which may relate to the reaction of autoantibodies with a podocyte antigen, to form immune complexes in situ [3]. Such in situ formation in the sub-epithelial space of the glomerular basement membrane is responsible for the functional impairment of the glomerular capillary wall, which causes proteinuria. The heterogeneity of membranous nephropathy and a lack of reliable biomarkers make the treatment of IMN controversial and challenging [4].

Phospholipase A_2 receptor (PLA_2R) is a type I transmembrane glycoprotein of 180-200 kDa related to the C-type animal lectin family that includes the mannose receptor [5, 6]. Group IB secreted PLA_2 ($sPLA_2$-IB) acts as an endogenous PLA_2R ligand, and when binding to the receptor it will elicit a variety of biological responses,

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including cell proliferation, cell migration, hormone release, and eicosanoid production [7]. More recently, PLA_2R was reported to be the target antigen of autoantibodies in adults with IMN [8]. The use of anti-PLA_2R as a sensitive and specific marker for IMN has been extensively evaluated. However, there is discrepancy in the results regarding the relationship between anti-PLA_2R level and clinical presentation. Thus, a systematic review and meta-analysis were performed to investigate the diagnostic values of anti-PLA_2R level for IMN.

Materials and methods

Search strategy

Medline/PubMed, Embase, and Cochrane databases were searched from inception until June 30 2013 to identify eligible studies using the IMN-related terms "idiopathic membranous nephropathy" and "primary membranous nephropathy" combined with the term "phospholipase A_2 receptor". The reference lists of the identified articles were also reviewed manually to identify additional articles.

Data extraction and quality assessment

Two reviewers (S.H. and D.W.) independently extracted data from all preliminary studies fulfilling the eligible criteria. Disagreement was discussed and settled using a third opinion (J.C.). The extracted information included: time and method of PLA_2R measurement, sample size, age, sex, country, biological material in which PLA_2R was measured (serum or biopsy), sensitivity, and specificity. The numbers of true-positive, false-positive, false-negative, and true-negative results in each included study were calculated.

One author independently assessed the quality of the included studies using the guidelines of the updated Quality Assessment of Diagnostic Accuracy Studies (QUADAS)-2 tool [9]. This revised tool is a considerable improvement from the original tool as it allows for more transparent rating of bias and applicability of preliminary diagnostic accuracy studies.

Statistical analysis

All of the data were entered into the Meta DiSc 1.4 (Meta Analysis for Diagnostic and Screening Trials) software for analysis. Sensitivities, specificities, diagnostic odds ratios (ORs), or relevant 95 % confidence intervals (CIs) estimated for each study were combined across studies using a random effects model. Between-study heterogeneity of sensitivities and specificities was tested using the Mantel–Haenszel Chi squared test with n-1 degrees of freedom (n is the number of studies). Statistical hypotheses (2-tailed) were tested at the significance level of 5 %.

Results

Search results and study characteristics

The preliminary search based on abstract and/or title yielded 125 articles, of which 104 were excluded because they were reviews, or irrelevant to the present analysis. Of the remaining 22 articles, after reading the full text, 12 were excluded because not focused on IMN (10 articles dealt with gene polymorphisms while 2 were investigating the diagnostic value of PLA_2R level in recurrent and *de novo* membranous nephropathy). Finally, 10 studies [8, 10–18] were included in the analysis (Fig. 1).

The characteristics of the included studies are listed in Table 1. All studies were published in English. They consisted of 6 prospective cohort studies [8, 10, 11, 14, 16, 18] and 4 cross-sectional studies [12, 13, 15, 17]. The studies were published within a period of 5 years, and involved PLA_2R measurements at two stages of MN: the active stage and the remission stage. PLA_2R was measured in both serum and biopsy in two studies [8, 13], only in serum in seven studies [10–12, 14, 15, 17, 18], and only in biopsy in one study [16]. PLA_2R was measured by western blotting in three studies [8, 11, 17], by immunofluorescence in five studies [10, 12, 13, 16, 18], and by enzyme-linked

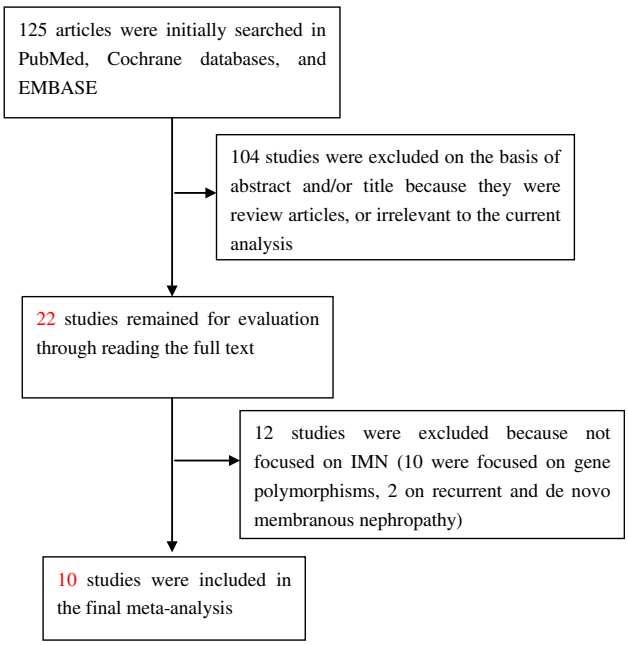

Fig. 1 Flow chart of study selection. *IMN* idiopathic membranous nephropathy

Table 1 Characteristics of included studies

References	Country	Age (years)	Mals (%)	Sample size	PLA_2R measurement	PLA_2R assay	Blinding of investigators
Beck [8]	England	NR	NR	89	Serum + biopsy	Western blotting	Yes
Hoxha [10]	Germany	NR	NR	360	Serum	Immunofluorescence	NR
Qin [11]	China	NR	NR	106	Serum	Western blotting	NR
Ben	Australia	NR	NR	68	Serum	Immunofluorescence	NR
Svobodova [13]	France	53.8	72.3	84	Serum + biopsy	Immunofluorescence	NR
Behnert [14]	USA	NR	NR	265	Serum	IIF-CBA	Yes
Kanigicherla [15]	England	54	71.0	163	Serum	ELISA	Yes
Larsen [16]	USA	57.5	65.9	165	Biopsy	Immunofluorescence	NR
Oh [17]	Korea	54.7	53.0	123	Serum	Western blotting	No
Hofstra [18]	The Netherlands	NR	77.8	117	Serum	IIFT and ELISA	NR

IIF-CBA indirect immunofluorescence cell based assay, *ELISA* enzyme-linked immunosorbent assay, *IIFT* indirect immunofluorescence testing, *NR* not reported

Table 2 Quality assessment of individual studies

Studies	Risk of bias				Applicability concerns		
	Patients selection	Index test	Reference standard	Flow and timing	Patient selection	Index test	Reference standard
Beck [8]	↑	↑	↑	?	↑	↑	↑
Hoxha [10]	↑	↑	↑	↑↑	↑	↑	↑
Qin [11]	↑	↑	↑	↑	↑	↑	↑
Ben 2012	↑	↑	↑	↑↑	↑	↑	↑
Svobodova [13]	↑	↑	↑	↑	↑	↑	↑
Behnert [14]	↑	↑	↑	↑↑	↑	↑	↑
Kanigicherla [15]	↑	↑	↑	↑	↑	↑	↑
Larsen [16]	↑	↑	↑	↑↑	↑	↑	↑↑
Oh [17]	↑	↑	↑	↑	↑	↑	↑
Hofstra [18]	↑	↑	↑	↑	↑	↑	↑

↑ low risk, ↑↑ high risk, ? unclear risk

immunosorbent assay (ELISA) [15] and indirect immunofluorescence cell-based assay (IIF-CBA) [14], each in one study.

Quality assessment

The quality assessment of all included studies based on QUADAS-2 is shown in Table 2. All studies used a convenience sample, but the blinding of investigators was documented only in three studies [8, 14, 15]. Six [8, 10, 11, 14, 16, 18] of the nine studies were prospective, and four studies [12, 13, 15, 17] were retrospective in design.

Diagnostic value of serum anti-PLA_2R in IMN prediction

The diagnostic values of serum anti-PLA_2R level in predicting IMN at the active stage and the remission stage were investigated in nine studies [8, 10–15, 17, 18]. One study [13] was excluded because of too few people in the control group. As a result, a total of eight studies (9 data points because results for indirect immunofluorescence testing and ELISA were reported separately for the Hofstra study [18]). Across all settings, the estimated sensitivity was 69.0 % (95 % CI 65.0–72.0) and specificity was 99.0 % (95 % CI 98.0–99.0), with a diagnostic OR of 247.41 (95 % CI 67.51–906.69; $p = 0.0198$). Figures 2 and 3 show forest plots with the pooled sensitivities and specificities based on patients at different stages.

Three [11, 15, 17] of 7 studies provided the diagnostic value of serum anti-PLA_2R level in predicting IMN at active stage, with a sensitivity of 74.0 % (95 % CI 67.0–80.0), a specificity of 95.0 % (95 % CI 91.0–98.0), and a diagnostic OR of 54.22 (95 % CI 19.28–152.45; $p = 0.2404$). There was moderate heterogeneity between

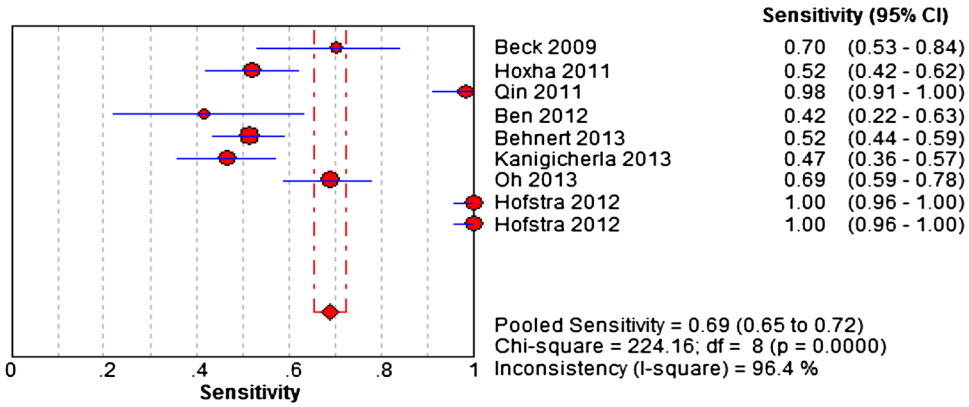

Fig. 2 Forest plot showing the pooled sensitivities of serum anti-PLA_2R level in patients at two stages (active and remission)

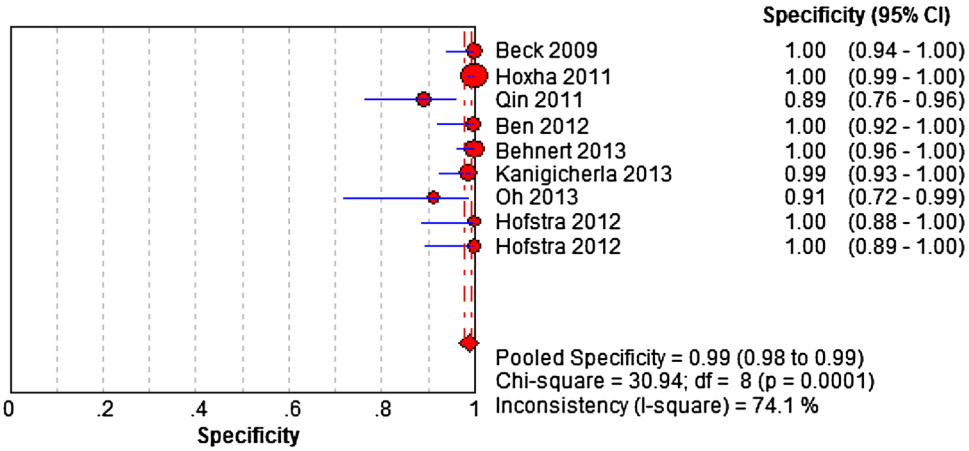

Fig. 3 Forest plot showing the pooled specificities of serum anti-PLA_2R level in patients at two stages (active and remission)

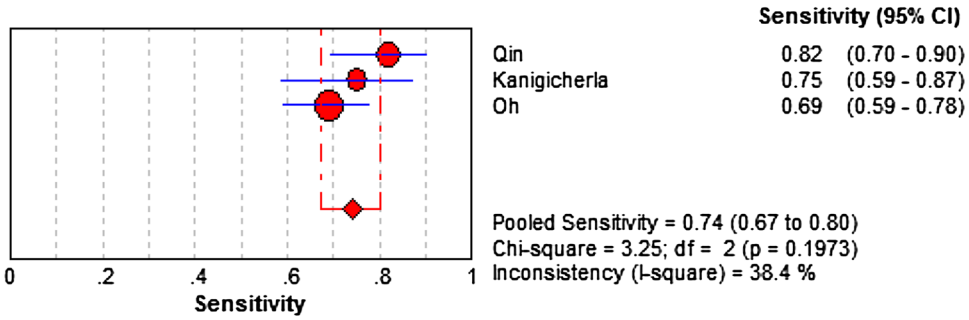

Fig. 4 Forest plot showing the pooled sensitivities of serum anti-PLA_2R level in patients at at the active stage

studies as evidenced by an I^2 of 29.8 % and Q test $p = 0.2575$.

Diagnostic value of biopsy PLA_2R antigens in IMN prediction

The diagnostic values of PLA_2R antigens of kidney biopsies in predicting IMN at two stage were investigated in three studies [8, 13, 16]. However, only two studies [13, 16] provided complete data for the meta-analysis. The estimated sensitivity was 73.0 % (95 % CI 65.0–80.0) and specificity 83.0 % (95 % CI 74.0–90.0), with a diagnostic OR of 13.75 (95 % CI 7.11–26.62; $p = 0.8185$). Figures 4 and 5 show forest plots with the pooled sensitivities and specificities based on patients at the active stage.

One study [13] provided the diagnostic value of biopsy PLA_2R antigens in predicting IMN at the active stage. The number of true-positive, false-positive, false-negative and true-negative was 23, 3, 5, and 16, respectively. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of biopsy of PLA_2R antigens in predicting IMN at the active stage were 82.1, 84.2, 88.5, and 76.2 %, respectively.

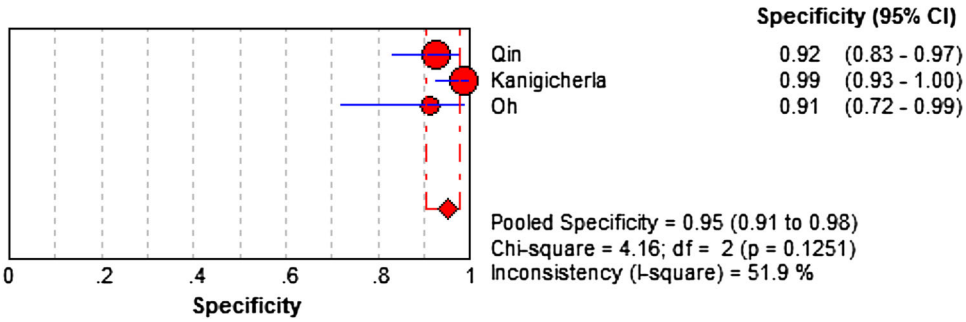

Fig. 5 Forest plot showing the pooled specificities of serum anti-PLA_2R level in patients at the active stage

Discussion

This systematic review and meta-analysis focused on the diagnostic value of PLA_2R for detection of IMN. First, serum anti-PLA_2R level was found to be a useful predictor of IMN, with a high diagnostic value for IMN (moderate sensitivity and high specificity at different stages, high sensitivity and high specificity at the active stage). Second, the level of PLA_2 antigens in kidney biopsy showed a good diagnostic value with high sensitivity and specificity for IMN at different stages.

PLA_2R is a 185-kDa glycoprotein that is expressed in lung macrophages [19], leukocytes [20], and glomerular podocytes [8]. But it is unknown why the pathologic features are limited to the kidney. Reportedly, autoantibodies against PLA_2R not only play a direct pathogenic role but also serve as sensitive and specific markers for IMN [8, 10, 11, 21, 22]. However, there is discrepancy in the results regarding the relationship between anti-PLA_2R level and the clinical presentation.

This analysis showed that serum anti-PLA_2R level does not perform as well for detecting IMN at different stages, with a diagnostic sensitivity of 0.69 (95 % CI 65.0–72.0), probably because anti-PLA_2R titres can affect at different stages. Reportedly in IMN patients, the level of serum anti-PLA_2R is related to the activity of IMN [23] and usually dramatically decreases in treatment responsive patients [21]. The prevalence of anti-PLA_2R in IMN patients who had not received immunosuppressive treatment was higher than in those who entered the remission stage. Moreover, anti-PLA_2R detected during initial diagnosis disappeared when the patients entered the remission stage, although autoantibodies were still persistently detected in non-remission patients [17]. In order to identify the relationship between anti-PLA_2R levels and clinical parameters of disease activity, we investigated the role of anti-PLA_2R in predicting active IMN. Findings showed that serum anti-PLA_2R level was of diagnostic value for active IMN, with a sensitivity of 0.74 (95 % CI 0.67–0.80). Therefore, we conclude that serum anti-PLA_2R level may perform as well for predicting IMN at the active stage as at remission stage. Because of the few included studies in the present meta-analysis, large cohort prospective studies on the relationship between anti-PLA_2R level and activity of IMN are needed.

Analysis showed that PLA_2R antigen in biopsy specimens was effective for detecting IMN at different stages, with a diagnostic sensitivity of 0.73. But only one study provided the diagnostic value of PLA_2R antigen of kidney biopsy in predicting IMN at the active stage. Though PLA_2R antigen in biopsy specimens was more sensitive than the serological test for the diagnosis of PLA_2R-related MN [13], we were unable to draw a similar conclusion. The reasons may be that: (1) the number of included studies is small; (2) the periods of PLA_2R measurement are different; (3) some patients had circulating anti-PLA_2R antibodies but did not have detectable PLA_2R in glomerular deposits [13, 24] while, on the contrary, some patients were negative for circulating anti-PLA_2R antibodies despite PLA_2R antigen positivity in the kidney biopsy [13]; (4) besides PLA_2R, there may be other target antigens, such as superoxide dismutase 2 (SOD_2) [25] and alpha enolase [26]. Due to these reasons, we were unable to conclude that the assessed PLA_2R antigen in biopsy specimens was superior to serological test for diagnosis of IMN. Further investigations should focus on comparing the diagnostic value of PLA_2R in kidney biopsy with circulating anti-PLA_2R antibodies in larger cohorts in prospective protocols.

One major limitation of our analysis is the heterogeneity of across-study sensitivity ($I^2 = 96.4$ %), probably due to the small number of studies, small sample size, low quality of the studies as assessed by QUADAS-2, different periods of PLA_2R measurement, and different assays used to assess the predictive value of PLA_2R. Then subgroup analysis was performed to further explore the sources of heterogeneity, and the measurement time was found to be a possible explanation. Early serum anti-PLA_2R level or PLA_2R antigens in biopsy specimens (at active stage) was of diagnostic value in predicting IMN, which is useful in clinical settings.

In conclusion, the present meta-analysis demonstrates that serum anti-PLA_2R level is of diagnostic value for IMN

in the active stage. But more properly designed investigations should be performed to reveal the diagnostic value of circulating anti-PLA_2R antibodies versus PLA_2R antigens in kidney biopsy. The included studies were nonrandomized and potential confounders could not be strictly controlled. The final conclusion about the appearance of anti-PLA_2R levels in patients with primary MGN can, however, only be confirmed by a large-cohort prospective study, which considers the degree of proteinuria, immunosuppressive treatment, time of observation, and repetitive measurements of anti-PLA_2R levels.

Conflict of interest The authors report no conflict of interest or relevant disclosures.

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