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Anti-phospholipase A_2 receptor antibodies in the diagnosis of idiopathic membranous nephropathy

Anticorpo anti recetor da fosfolipase A_2 no diagnóstico de nefropatia membranosa idiopática

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ABSTRACT

Circulating anti-phospholipase A_2 receptor antibodies (anti-PLA_2R) have been described in 70% to 80% of the patients with idiopathic membranous nephropathy (iMN), but not in patients with secondary membranous nephropathy or other glomerular diseases. The goal of this study was to evaluate the sensitivity and specificity of the assay for anti-PLA_2R in the diagnosis of iMN. Anti-PLA_2R IgG, Elisa and immunofluorescence tests were used to detect circulating anti-PLA_2R. These tests were applied in 53 patients who had a kidney biopsy. Of these, 38 had histological diagnosis of membranous nephropathy (MN) and the remaining had other glomerular diseases. The MN was classified as idiopathic in 33 patients after clinical exclusion of secondary causes. Anti-PLA_2R were positive in 57.6% of the patients with iMN. All patients with secondary membranous nephropathy or other glomerular diseases did not show circulating anti-PLA_2R. The sensitivity was 57.6% (CI 39.2-74.5) and specificity 100% (CI 47.8-100), AUC 0.788; $p < 0.0001$ for the detection of iMN. 71.4% of the iMN patients that tested negative for anti-PLA_2R were in partial or complete remission. The detection of anti-PLA_2R in the studied population had a specificity of 100% for the iMN diagnosis. Prior treatments seem to make the test negative and contribute to a lower sensitivity.

Key-Words: Anti-phospholipase A2 receptor; idiopathic membranous nephropathy; nephrotic syndrome; podocyte.

RESUMO

Anticorpos circulantes anti recetor da fosfolipase A_2 (anti-$RFLA_2$) têm sido descritos em 70% a 80% dos doentes com nefropatia membranosa idiopática (NMi) mas não em doentes com nefropatia membranosa secundária ou outras doenças glomerulares. O objetivo deste estudo foi avaliar a sensibilidade e

especificidade do teste para anti-$RFLA_2$ no diagnóstico de NMi. Foram utilizados os testes Elisa e imunofluorescência para deteção do Anti-$RFLA_2$, IgG. Estes testes foram aplicados a 53 doentes com biópsia renal. Destes, 38 tiveram o diagnóstico histológico de nefropatia membranosa (NM), enquanto os restantes 15 doentes apresentaram outras doenças glomerulares. A NM foi classificada como idiopática em 33 doentes, após exclusão de causas secundárias. O Anti-$RFLA_2$ foi positivo em 57,6% (n = 19) dos doentes com NMi. Todos os doentes com nefropatia membranosa secundária ou outras doenças glomerulares não apresentaram anti-$RFLA_2$. A sensibilidade foi de 57,6% (IC 39,2-74,5) e a especificidade de 100% (IC 47,8-100), AUC 0,788; $p < 0{,}0001$ para a deteção de NMi. 71,4% (n = 10) dos 14 doentes com NMi que tiveram teste negativo para anti-$RFLA_2$ estavam em remissão parcial ou completa. A deteção do anti-$RFLA_2$ na população estudada apresentou uma especificidade de 100% para o diagnóstico de NMi. O sucesso do tratamento prévio terá contribuído para a baixa sensibilidade.

Palavras-Chave: Anti recetor da fosfolipase A_2; nefropatia membranosa idiopática; podócito; síndrome nefrótico.

INTRODUCTION

Membranous nephropathy (MN) is the most common cause of nephrotic syndrome in adults, with up to one-third of patients progressing to end-stage renal disease[1,2].

This is an auto-immune disease that affects the kidney glomerulus, resulting from the formation of immune deposits on the outer regions of the glomerular basement membrane. This disease is classified as idiopathic when any identified aetiology is excluded, or secondary if caused by other clinical conditions.Recently the identification of several antigens has helped understanding the molecular bases of MN[3,4].

The M-type phospholipase A_2 receptor (PLA_2R) was identified as autoantigen in the idiopathic membranous nephropathy (iMN). This is located in the glomerular capillary wall at the podocyte cell membrane[5].

The phospholipase A_2 receptor (PLA_2R) is a type I transmembrane glycoprotein related to the C-type animal lectin family that includes the mannose receptor. PLA_2R regulates a variety of biological responses elicited by specific types of secretory phospholipase A_2 ($sPLA_2s$). Group IB $sPLA_2$ ($sPLA_2$-IB) acts as an endogenous PLA_2R ligand that induce responses like cell proliferation, cell migration, and lipid mediator production[6].

Circulating anti-phospholipase A_2 receptor antibodies (anti-PLA_2R) has been described in 70% to 80% of the patients with iMN, but not in patients with other glomerular diseases and rarely in secondary membranous nephropathy[7]. The use of anti-PLA_2R has been evaluated, however, doubts remain about whether it is a sensitive and specific marker for iMN.

The goal of this study was to evaluate the sensitivity and specificity of the assay for anti-PLA_2R in the diagnosis of iMN.

SUBJECTS AND METHODS

The population was chosen among patients (n = 53) followed in Nephrology Clinic, with nephrothic syndrome/nephrotic proteinuria and histological diagnosis by kidney biopsy.

Anti-PLA_2R IgG, Elisa and immunofluorescence tests were used to detect serum circulating anti-PLA_2R. The tests were applied prospectively for 18 months (November 2011 to May 2013).

Secondary causes of MN were excluded after clinical workup including detailed medical history, clinical examination, analysis like lupus antibodies, hepatitis B, C and HIV, and at least chest X-ray and abdominal ultrasound.

The Fisher exact test was used to compare variables. A ROC curve was used to determine the sensitivity and specificity of the assay, and $p < 0.05$ was considered as statistically significant. Medcalc 12.4.0 was used as statistical software.

RESULTS

The results of biopsies of the 53 patients evaluated are listed in Table I. Of these, 38 had histological diagnosis of membranous nephropathy and the remaining 15 patients had other glomerular diseases. We did not find histological differences between idiopathic and secondary MN. The MN was idiopathic after clinical exclusion of secondary causes in 33 patients. The characteristics of iMN patients are listed in Table II.

Anti-PLA_2R was positive in 57.6% (n = 19) of the 33 patients with iMN. All patients with secondary membranous nephropathy or other glomerular diseases did not show circulating anti-PLA_2R (Table III). The sensitivity was 57.6% (CI 39.2-74.5) and specificity 100% (CI 47.8-100), AUC 0.788; $p < 0.0001$ for the detection of iMN (Fig. 1).

Table I

Description of biopsies results

Membranous nephropathy (MN) n = 38	**Idiopathic MN (iMN) n = 33**	
	Secondary MN (sMN) n = 5	Solid neoplasia n = 3
		Lupus nephritis n = 2
Other Glomerular Diseases (other GD) n=15		Minimal change disease n = 2
		Focal and segmental glomerulosclerosis n = 7
		Post-infectious glomerulonephritis n = 1
		Diabetic nephropathy n = 2
		Calcineurin inhibitors toxicity n = 1
		Kambham disease n = 1
		IgA Nephropathy n = 1

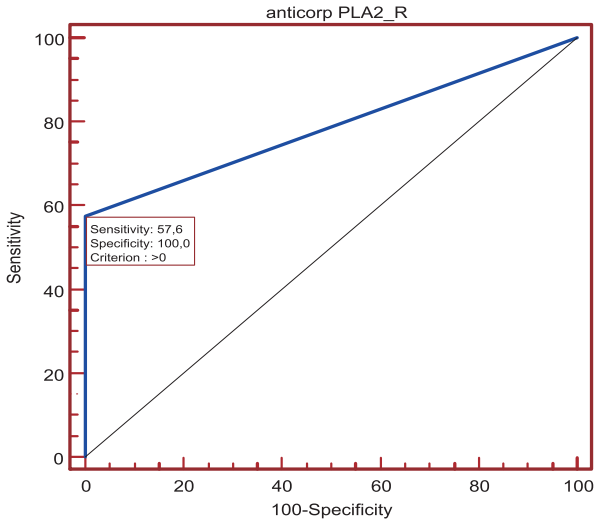
Table II

Characteristics of iMN patients

Number of patients	33
Age (mean)	52
Gender (m:f)	26:7

Figure 1

ROC curve



Ten (71.4%) of the 14 iMN patients with negative test for anti-PLA_2R were already in treatment when the test was done and were in partial or complete remission of the disease (Table IV). In three patients the test was repeated and we observed that Anti-PLA2R became negative in two patients with iMN after treatment with cyclosporine and Anti-PLA_2R became positive in one patient with relapse.

Table III

Circulating PLA2R antibodies in the studied population

	iMN (n = 33)	sMN (n = 5)	Other GD (n = 15)
Anti-PLA2R positive	19	0	0
Anti-PLA2R negative	14	5	15

Table IV

Circulating PLA2R antibodies in iMN patients

Anti-PLA2R positive 57.6%	Active disease	19
	Remission	0
Anti-PLA2R negative 42.4%	Active disease	4
	Remission	10

DISCUSSION

Anti-PLA_2R in the studied population was only detected in the serum of patients with idiopathic MN and was negative in all patients with secondary MN or with other glomerular diseases. The high specificity of this test can help to make a diagnosis in patients with nephrotic syndrome/nephrotic proteinuria when a kidney biopsy is not performable. A positive test in these patients is highly suggestive of an idiopathic MN as the cause of the disease[2,3].

Our study demonstrated a low sensitivity for idiopathic MN diagnosis (57.5%). We think that this result was the consequence of some patients being already in treatment when the test was done. Some authors showed reduction of the antibody levels with treatment[8,11]. It seems that the treatment and the disease remission are associated to a negative test in patients with idiopathic MN proven by biopsy and a negative clinical evaluation.

This test seems to be a useful predictor of iMN with a high diagnostic value for iMN at the active stage. Other studies also showed high specificity in predicting IMN (between 91% and 100%) and a lower sensitivity for both active and remission stages (between 47% and 69%)[9-11,13]. Three patients had successive determinations of Anti-PLA_2R. This test became negative in two patients with iMN after treatment with cyclosporine. Anti-PLA_2R became positive in one patient with relapse. The usefulness of this assay as a treatment response marker should be evaluated.

All patients with sMN tested negative. The test proved consistently negative in patients with lupus nephritis[12]. These findings strongly suggest that lupus MN is not related to PLA2R. All in all, large-cohort prospective studies are required, integrating the degree of proteinuria, immunosuppressive treatment, time of observation, repetitive measurements of anti-PLA2R levels and glomerular immune deposits of anti-PLA2R[13,14].

Conflict of interest statement: None declared

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