ANTI-PHOSPHOLIPASE A2 RECEPTOR ANTIBODIES - DIAGNOSTIC RELIABILITY FOR DIAGNOSING PRIMARY MEMBRANOUS NEPHROPATHY

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Abstract: The etiology of membranous nephropathy (MN) remains incompletely understood. As a specific glomerular disease, it manifests itself in about 80% of cases as primary membranous nephropathy (PMN). According to Roncko et al., in other cases, the causes of the disease may be of a different nature, such as hepatitis infection, autoimmune disease, etc. (2021). In these cases, it is referred to as secondary (SMN). In a review of the scientific literature, data indicate that biopsy of kidney is used as the "gold standard" for making a diagnosis MN (Kalantari et al., 2017; Lai et al., 2015). The method is also invasive and, according to Dimitrios-Anestis et al. (2009), is often accompanied by complications of various kinds, including pain or bleedin. In rarer cases, according to Parish (1992), it may be accompanied by other infectious complications, as well as improperly punctured other tissue. The role of autoantibodies binding the phospholipse A2 receptor (PLA2R) in the clinical management of MH has been emphasized in recent years. Therefore, detection of these antibodies is thought to make them specific for the diagnosis of PMN (Li et al., 2022) and an important diagnostic tool (Netti et al., 2019) for monitoring the disease and assessing the response to treatment (Obrisca et al., 2015). Authors such as Hofstra et al. (2012) and Hoxha et al. (2014) point out the need to implement new clinical laboratory methods for quantitative determination of this indicator, which are easy to perform and reliable. Data from the scientific literature show that in clinical laboratory practice for determining antibodies against PLA2R there are qualitative, semi-quantitative or quantitative methods. The goal we set for our study is to determine the diagnostic reliability of the concentration of antiphospholipase A2 receptor antibodies (anti-PLA2R) in serum of patients with PMN and also their cut-off value. A total of 233 subjects were studied in this study - healthy subjects and patient groups. The patient group included 52 with PMN, 12 with SMN, 49 with other nephropathies (ON) and 120 clinically healthy subjects (HC). To determine a serum concentration of anti-PLA2R autoantibodies, an ELISA kit (Anti-PLA2R ELISA, IgG, EUROIMMUN, Lübeck, Germany) was used, and the results were read using an MR-96A microplate reader from MINDRAY. The obtained results were statistically processed, which allowed the calculation of criteria a diagnostic reliability about the anti-PLA2R antibodies indicator. We also determined the cut off value of a test using a receiver operating characteristic (ROC) curve constructed by us. Data analysis was statistically processed using the Med Cal cv.18.5 program, 2018 Med Cal c Software. ELISA method for a quantitative determination of anti-PLA2R antibodies has shown good diagnostic reliability. This allows it to be introduced into clinical laboratory practice and its routine use. We received the cut-off value 19.84 RU/ml. This value allows the diagnosis of PMN and can be used to distinguish these patients from other forms of MN. All these characteristics of the ELISA method determine its preference in its application in routine clinical laboratory practice.
Keywords: membranous nephropathy, anti-phospholipase A2 receptor antibodies, diagnostic reliability

1. INTRODUCTION

MN is an autoimmune glomerular disease, the etiology of which is still not fully understood. It is known that primary forms of the disease are in about 80% of cases. The remaining 20% are forms of SMN, the etiological causes of which may be due to various causes related to diseases with autoimmune genesis, diseases of an infectious or malignant nature and others. PLA2R is believed to be a major target antigen in PMN, accounting for about 80% of the disease. In our study, we set ourselves a goal to determine a diagnostic reliability as well as cut-off value for anti-PLA2R antibodies in serum of patients with primary form of the disease.

2. MATERIALS AND METHODS

The study design included 233 patients who were divided into different groups: PMN, BMN, and ON patients. HC (n = 120) were used as a control group.

All participants in the study were divided in a two groups:

- Positive for PMN, coded as1–included patients positive for PMN
- negative for PMN, coded as 0 – included healthy individuals and patients without PMN

An ELISA kit was used to quantify serum anti-PLA2R antibodies, and the concentration was read on a microplate reader.

3. RESULTS

In four-cell table 1, the distribution data are presented.

Using statistical analysis, the true positive (TP), true negative (TN), false positive (FP) and false negative (FN) rates were calculated. The data are presented in four-cell table 1.

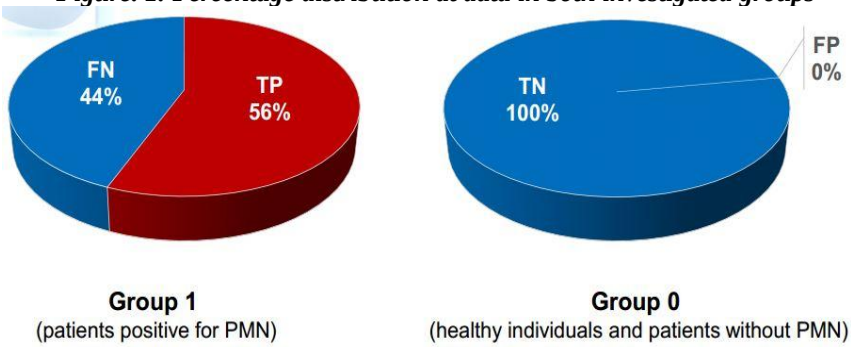
Table. 1. Data presentation for TP, TN, FP and FN in four-cell table

		Illness +	Illness –
New parameters	+	TP (n = 29)	FP (n = 0)
	–	FN (n = 23)	TN (n = 181)

Source: Author's research

The calculated data in percentages for group 0 and group 1, as well as their distribution, are presented in a figure 1.

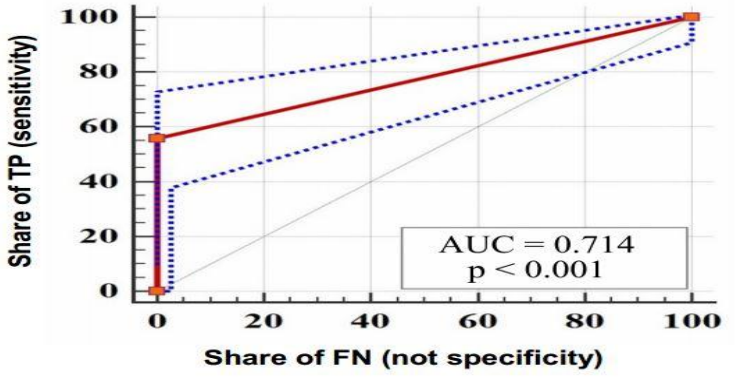
Figure. 1. Percentage distribution at data in both investigated groups



Source: Author's research

We constructed a ROC-curve, with the help of which we determined the cut off value of anti-PLA2R (fig.2). This allowed us to distinguish the presence or absence of PMN.

Figure. 2. ROC-curve of anti-PLA2R1 (red continuous line) and its 95% confidence interval (blue dashed line)



Source: Author's research

The abscissa is marked with the frequency of false positive results, and the frequency of true positive results –with the ordinate. The calculated area under the curve (AUC) is 0.714 (table.2).

Table. 2. Data from ROC curve analysis

Indicator	AUC ± SE	P	Lower limit (95% CI)	Upper limit (95% CI)
Anti-PLA2R1 (RU/ml)	0.714 ± 0.05	0.0001**	0.614	0.815

****Area Under the Curve (AUC), significance level – P, SE – standard error, Confidence Interval (CI)*

Source: Author's research

The obtained data on a diagnostic reliability of antiPLA2R1and the cut-off value are presented in Table3.

Table. 3. Diagnostic reliability criteria for the anti-PLA2R antibody indicator

Indicator	Sensitivity	Specificity	Diagnostic efficiency	PPV	NPV	Cut-off value (95% CI)
Anti-PLA2R1 (RU/ml)	56% (41.33–69.53)	100% (97.98–100)	90% (88.18–91.82)	100%	88.73%	19.84 (19.19–19.84)

PPV – positive predictive value, NPV – negative predictive value

Source: Author's research

4. DISCUSSIONS

In this study, we aimed to investigate a diagnostic reliability indicators of the results obtained for anti-PLA2R antibodies. The study included a 231 participiens from different groups. These groups were represented by healthy individuals as well as patients with PMN, SMN and ON. The results for the diagnostic reliability criteria of the anti-PLA2R antibodies indicator that we obtained are as follows: 56% sensitivity; 100 % specificity; 90% diagnostic efficiency; 100% PPV and 89% NPV. We constructed a ROC curve and determined AUC 0.714, also a cut off value 19.84 RU/ml. The results obtained allowed us to distinguish patients with PMN from those negative for this disease. The cut off value declared by the company manufacturing the test kit is (20 RU/ml) and very close to the one we obtained. Also, at this cut off value that we indicated, we determined a specificity of anti-PLA2R antibodies that completely matches that stated by the test kit manufacturer (specificity is 100%).

A scientific paper published by McDonnell et al. (2023) points out the revolutionary role of anti-PLA2R antibodies in understanding the etiology and treatment of MH. According to them, recent studies challenge the need for renal biopsy in the diagnosis of this disease, as these antibodies have high sensitivity (75%) and specificity (100%).

Porcelli et al. (2020) in their study conducted among a large Italian multicenter cohort reported sensitivity and specificity data that are similar to ours. The disease in patients with PMN is proven by kidney biopsy, as in patients with other kidney diseases. The authors from study aim for determine the optimal cut off by which it would be possible to distinguish positive results for PMN from negative ones. Their study included 495 patients, divided into two groups: 126 of the total group with PMN and 396 without this disease. All patients in the study, as well as those in ours, were tested before the administration of immunosuppressive therapy. Values for a diagnostic reliability of their results were established as follows: 61% sensitivity, 100% specificity, 90% DE – 90%, 100% PPV and 89% NPV. The results obtained from the post-constructed ROC curve show an AUC of 0.938. They obtained values for PPV and NPV compared to ours show very similar results: 100% vs. 99% and 89%, respectively. Also, the PPV and NPV values are very close to ours: 100% vs. 99% and 89% vs. 88%, respectively. Some of the criteria show slight differences. For example, their sensitivity obtained by them (61%) compared to ours (56%) is slightly higher. The same trend is observed in the measured area under the curve (0.938) compared to our obtained one (0.714).

Provatopoulou et al. conducted a retrospective study in a Greek population in 2019. The total patient group in this study was n = 59. The study design included two groups of patients: 33 with PMN and 26 without PMN.

A cut-off value of 20 RU/mL was used. Their results showed that our sensitivity (56%) was higher than theirs (48.5%). The study lacks data on the remaining criteria for diagnostic reliability of the indicator. Using a lower cutoff value of 2 RU/mL compared to that recommended by the kit manufacturer was found to result in higher sensitivity (58%).

Hihara and et al. (2016) and Katsumata et al. (2020) independently conducted clinical studies among Japanese patients with the same disease. Their results were similar to ours: sensitivity (50.00% and 52.17%) and specificity (100%), PPV (100%) and different for NPV (53% vs. 74%). In our study, the sensitivity values (56%) are slightly

higher than theirs (50.00% and 52.17%). Similar results were obtained for NPV where ours (89%) are higher than theirs (53% and 74%). No data on DE and AUC were published in the article. From the summarized data obtained in our study, we can conclude that the ELISA method we used for determining serum anti-PLA2R antibodies has good diagnostic reliability. The results showed diagnostic specificity – 100% and PPV – 100%, accompanied by good DE – 90% and NPV – 89%. Data on a diagnostic sensitivity and diagnostic efficiency for our methodological studies allow the results for anti-PLA2R antibodies to be used in clinical practice for the diagnosis of this severe and difficult-to-treat disease, the treatment of which is accompanied by serious side effects.

McDonnell

5. CONCLUSIONS

In our opinion, the quantitative ELISA method for the quantitative determination of anti-PLA2R antibodies has good characteristics and diagnostic reliability criteria. The method can be routinely applied in clinical laboratory practice. We obtained a cut-off value that can distinguishing patients with PMN from other forms of MH.

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