SERUM ANTI-THROMBOSPONDIN TYPE-1-DOMAIN CONTAINING 7A PROTEIN ANTIBODIES - DIAGNOSTIC RELIABILITY

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Abstract: Various researchers have defined membranous nephropathy (MN) as a disease of autoimmune origin (Ronco et al., 2021; Dantas et al., 2023). The probable mechanism by which it occurs is the binding of circulating autoantibodies to podocyte antigens expressed by renal glomeruli. This leads to the formation of electron-dense immune complexes with subsequent activation of the complement system. Clinically disease presents with proteinuria in the nephrotic range and edema (Hoxha et al., 2022). The disease can evolve into severe thrombotic complications or the development of end-stage renal failure. Over the past twenty years, great progress has been made in discovery of probable antigens, being the protein "M-type phospholipase-A2 receptor". According to Radhakrishnan et al. (2024), it is a transmembrane receptor that is expressed on the surface of podocytes. Due to loss of immune tolerance by mechanisms that, according to the author, are not yet fully understood, antibodies are formed against this autoantigen, mainly of the immunoglobulin G4 class. This likely mechanism of injury is responsible for 70% to 80% of primary MH cases. The study of the amount of allows us to assess the disease and its activity and prognosis. In 2014, Tomas et al. reported their discovery of a novel antigen, called thrombospondin type-1 domain containing 7A protein (THSD7A), which they believe is a novel target antigen responsible for the pathogenesis of primary membranous nephropathy (PMN). Autoantibodies directed against THS7DA are predominantly of the IgG4 class, which have been observed using immunofluorescence microscopy of kidney biopsy preparations. Various groups of authors (Hoxha et al., 2017; Hanset et al., 2020) report that THSD7A-associated MN has a higher incidence (20% to 50%). Currently, testing for antibodies against THSD7A (anti-THSD7A) is poorly used in clinical laboratory practice. Data on in scientific literature are quite scarce. According to Tomas et al. (2016) primary membranous nephropathy is still not fully understood. We studied a total group of 134 individuals, which was composed of patients with kidney diseases n = 84 and the control group of healthy individuals n = 50. The renal disease group included three subgroups of patients: 23 PMN patients who were negative for anti-PLA2R antibodies, 12 with secondary membranous nephropathy (SMN) and 49 with other nephropathies (ON). EUROIMMUN indirect immunofluorescence assay (IIFT) was used serum titer. The obtained data were statistically processed. The summary results for the anti-THSD7A antibody marker showed very good diagnostic reliability. This allows us to determine the titer of these circulating antibodies in patients with PMN. In conclusion, we can say method for determining has good diagnostic reliability.
Keywords: membranous nephropathy, primary membranous nephropathy, phospholipase receptor antibodies

1. INTRODUCTION

According to literature data importance quantitative in has been increasing in the last few years. In 2014, Tomas et al. reported a new target antigen in PMN diagnostics called THSD7A. Initially, immunoblot analysis (Western blot) was used to determine. Our aim in this study is reliability of the anti-THSD7A assay.

2. MATERIALS AND METHODS

When determining diagnostic reliability, we apply the following sequence:

1. Clarification of the two groups:

- negative for PMN (0) – includes healthy persons and patients without disease
- positive (1) – includes patients with proven PMN

2. Were included with following values.

In case of a positive sample, IgG antibodies from the sample bind to an antigen (THSD7A) expressed by the transfected test cells and an Ag-Ab complex is formed. Fluorescently labeled anti-human IgG (goat) antibody (Ab*) is added to each well. An Ag-Ab-Ab* complex is formed. Positive cells emit a borderline, fine-grained fluorescence. According to the manufacturer, no cross-reactivity with other antibodies was detected, such as patients positive for cANCA (n = 11), pANCA (n = 11), anti-GBM (n = 9) and HbsAg (n = 10). The linearity of the anti-THSD7A IIFT (IgG) was determined by serial dilution of patient samples. The correlation coefficient for all sera was > 0.95. Specified in reference limits recommended by the manufacturer are:

Titer 1: < 10 (IgG)

For the statistical processing of the obtained results and determination of diagnostic reliability, we used the MedCalc software program v. 18.5, 2018. All patients signed informed consent.

3. RESULTS

In shows the distribution of the data in four cells.

Table 1. Distribution of data for TP, TN, FP and FN values from the survey

		Illness +	Illness –
New parameters	+	TP (n = 2)	FP (n = 0)
	–	FN (n = 21)	TN (n = 111)

Source: Author's research

Summary data of the 2.

Indicator	Diagnostic sensitivity (95% CI*)	Diagnostic specificity (95% CI*)	DE (95% CI*)	PPV	NPV
Anti-THSD7A	8.69% (6.52–10.86)	100% (97.98–100)	84.33% (82.63–86.03)	100%	84.09%

CI - Confidence interval

Source: Author's research

The results obtained for the indicator anti-THSD7A antibodies listed in table 1

4. DISCUSSIONS

The clinical importance of determining serum concentrations of anti-PLA2R antibodies has increased in the past few years. Several years ago, Tomas et al. (2014) described THSD7A, which was suggested as a new target antigen for the diagnosis of MH. The WB method was used to determine the titer of anti-THSD7A antibodies. The authors describe it as reliable, but its performance is technically slow and its application is mainly for research purposes. A few years later, the company EUROIMMUN developed IIFT. As a new indicator, anti-THSD7A has not yet been established in clinical laboratory practice, which explains its lack of widespread application. Data from literature sources indicate that clinical studies aimed at evaluating the role of anti-THSD7A antibodies in PMN are few. Their role, according to Tomas et al. (2016) remains incompletely understood.

The goal we set was to investigate IIFT in determination. Total number of patients included in the study was 134 individuals. A prospective Lin groups whom 195 with 70 with non-PMN and 50 healthy controls. Their published data for 100% specificity coincide with ours. Their sensitivity was 1.47%, which is lower than ours (8.69%). Due to the lack of published data for the other criteria - DE, PPV and NPV, we were unable to make a comparison.

2017 determine diagnostic reliability anti-THSD7A antibodies was conducted among a group of 776 Chinese patients which included. The IIF method was used. The results for specificity and PPV are close to the criteria of they obtained (4.35%, 53, 66% and 52.81%, respectively).

In a clinical study conducted on average 2021 Brazilian patients the number studied was 59. The results in these patients were negative authors emphasize but It ranges for.

Despite small number of reports in the literature, from our obtained data of 100% diagnostic specificity and PPV, 84.33% DE and 84.09% NPV, we can conclude that the results for anti-THSD7A antibodies obtained by the IIF method show very good diagnostic reliability.

5. CONCLUSIONS

Our study contributes to enriching the data on IIFT characteristics are good, allowing detection.

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