

SERUM ANTIBODIES AGAINST PHOSPHOLIPASE A2 RECEPTOR – REFERENCE RANGES AND DIAGNOSTIC VALUE IN BULGARIAN PATIENTS WITH PRIMARY MEMBRANOUS NEPHROPATHY

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Abstract: The development of nephrotic syndrome in the disease membranous nephropathy is most often observed in adults. Literature data indicate that its pathogenesis is multifaceted. In a larger percentage of cases, they are defined as primary membranous nephropathy, which currently has an unclear etiology. It has been established that in the pathogenesis of primary membranous nephropathy, the main role is played by autoantibodies produced by the body against the phospholipase A2 receptor (anti-PLA2R1), which are expressed by human renal podocytes. Other antigens, such as thrombospondin type 1 domain-containing protein 7A and neural epidermal growth factor-like-1 protein, have also been associated with this disease, especially in cases with negative autoantibodies directed against the phospholipase A2 receptor. In recent years, a large number of new antigens associated with this disease have been discovered. However, in 10%-15% of cases, membranous nephropathy antigens remain unknown, and their pathogenesis remains unclear. Currently, interest in clinical laboratory practice directed at these antibodies is attracting increasing attention in terms of diagnosis and monitoring of the disease. Our study was conducted among a group of Bulgarian patients diagnosed with membranous nephropathy, which was proven with the help of a kidney biopsy. The goal we set was to determine the reference ranges for autoantibodies directed against the phospholipase A2 receptor among the Bulgarian population, as well as to determine the diagnostic and prognostic value of these antibodies. The study design included the study of a total of 233 individuals, of whom 120 were clinically healthy individuals and a group of 113 patients. The patient group included three groups - primary membranous (n =52), secondary membranous (n =12) and other (n = 49) nephropathies. The concentration of serum autoantibodies was determined using a kit for quantitative determination of serum antibodies against phospholipase A2 receptor from EUROIMMUN. The obtained values were read using a microplate reader. We obtained processed the determination reference ranges for the indicator antibodies against the phospholipase A2 receptor. A cutoff value of 19.84 RU/ml was also determined, which allows the diagnosis of primary membranous nephropathy, as well as the differentiation of these patients from other forms of membranous nephropathy. Therefore, our data confirm the usefulness of determining the serum concentration of these autoantibodies in the diagnosis. Their good correlation with the clinical condition of patients indicates the possibility of using them in clinical practice to monitor disease activity, select the right treatment, and predict the prognosis of the disease. The aim of analyzing the severity of the impact of autoantibodies directed against the phospholipase A2 receptor on clinical effectiveness is to improve the quality of life of these patients.

Keywords: primary membranous nephropathy; anti-phospholipase A2 receptor antibodies; reference ranges

1. INTRODUCTION

Membranous nephropathy (MN), defined as an autoimmune glomerular disease, currently has an etiology that is not yet fully understood. In about 80% of cases, it is defined as primary, as it (75-80%) is directed renal. In remaining ~ 20%, MN is defined as secondary. In this form of the disease, etiological causes can be different. According to Obriska et al. primary forms of the disease have a long natural course, which in its course shows heterogeneity, with exhibiting different showing end-stage renal failure (ESRF), and the rest experiencing spontaneous remission (2015). According to Svobodova et al., it is believed that the levels of these PLA2R1 antibodies found primary MN (PMN), which are absent in secondary forms (2013). There is evidence that changes in the anti-PLA2R1 titer precede the corresponding clinical changes by several months (Hofstra et al., 2011; Segara-Medrano et al. 2014). According to Ramachandran et al., quantitative determination of the concentration of these antibodies is gradually being introduced into current clinical practice for its application as a guide for diagnosis and treatment planning, as well as a proposal that in the near future they may replace renal biopsy (2017). The study conducted by us among Bulgarian patients set the goal of determining reference limits for this indicator, as well as studying anti-PLA2R1 in different groups of patients to determine the diagnostic value in making the diagnosis.

2. MATERIALS AND METHODS

The study of 233 patients aged 20 to 67 years for the period 2015 - 2018. The individuals included in were controls (HC) and patients with kidney diseases. The participants were previously informed about the purpose, as well as the expected results and benefits of the study of them

voluntarily. The inclusion criteria and, respectively, the exclusion criteria from this group are presented (table 1).

Table. 1. Criteria for selection of HC

Criteria for inclusion in the group of HC	Criteria for exclusion from the group of HC
1. Age over 18 years	1. Data on acute inflammatory diseases in the last 30 days
2. Volunteering	2. Chronic diseases (lung, liver, kidney, cardiovascular diseases)
3. Clinical health	3. Obesitas
4. Hematological laboratory parameters in reference intervals	4. Malignant diseases
5. Clinical and chemical laboratory parameters in reference intervals	5. Systemic diseases
6. Urine laboratory values in reference intervals	6. Surgical interventions in the last 3 months before blood collection
	7. Alcohol or drug abuse
	8. Heavy physical exertion or mental stress
	9. Pregnancy or lactation

Source: Author's research

The subdivision of patients with PMN and patients with secondary MN (SMN) was performed using the following criteria presented in Table 2.

Table. 2. Criteria for selection of patients with SMN

Clinical criteria	Pathomorphological criteria
1. Presence of paraneoplastic disease at diagnosis or within 5 years thereafter (Bacchetta et al., 2009; Dai et al., 2015)	1. Presence of mesangial and/or subendothelial immune deposits on immunofluorescence analysis (Ohtani et al., 2004; Lefaucheur et al., 2006)
2. Chronic HBV or HCV infection	2. Predominance of Ig A deposits (Mustonen et al., 1984)
3. Data on systemic diseases (systemic lupus erythematosus, rheumatoid arthritis)	3. Presence of mesangial proliferation

Source: Author's research

The third group, which includes patients with other nephropathies (ON), is presented in Table 3.

Table. 3. Composition of the ON group

Disease	Number of patients (n)
1. Minimal change disease	9
2. Tubulointerstitial nephritis	8
3. Diabetic nephropathy	17
4. Hypertensive nephroangiosclerosis	6
5. Light chain nephropathy	1
6. Membranoproliferative GN	2
7. Focal and segmental glomerular sclerosis and hyalinosis	3
8. Amyloidosis	1
9. Antiphospholipid syndrome	1
10. Membranous systemic lupus erythematosus	1

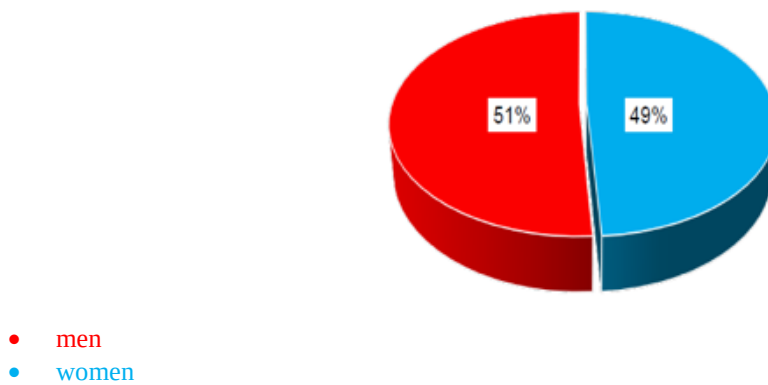
Source: Author's research

For quantitative determination of the concentration of anti-PLA2R1 antibodies in serum or plasma, an immuno-enzyme test kit. When interpreting results obtained, the values recommended by the manufacturer were used.

3. RESULTS

Recommended by International Federation of Clinical Chemistry (IFCC), the direct method was used to select healthy subjects for inclusion in the reference group (IFCC & CLSI EP28-A3c., 2010). Data from a study of 120 healthy subjects (59 males and 61 females) were used to determine the reference ranges for anti-PLA2R1 antibodies (figure 1).

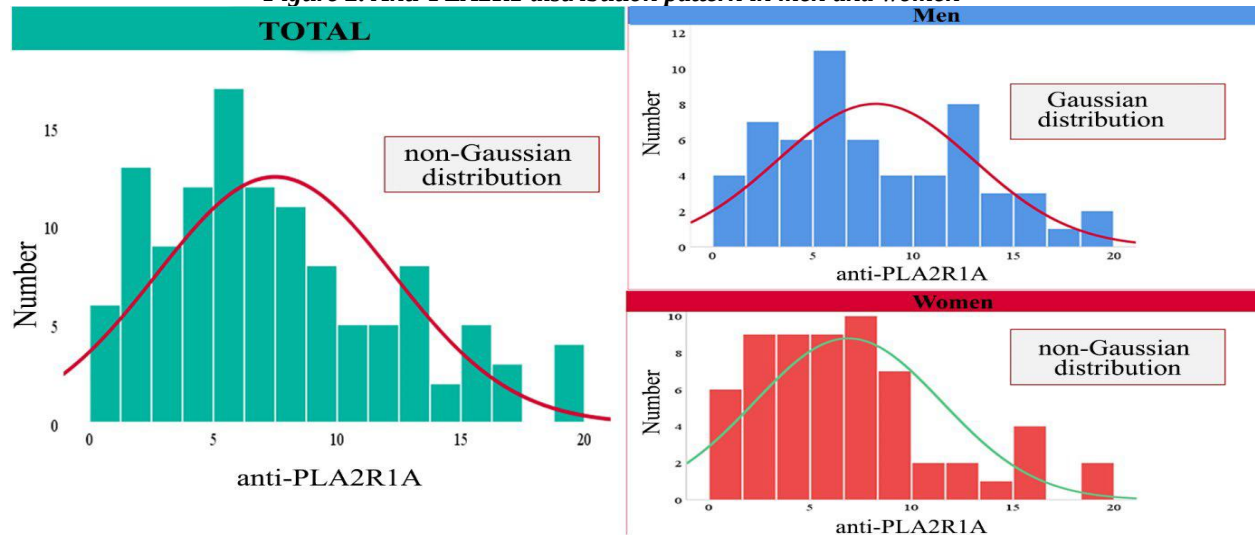
Figure 1. Distribution of participants in the reference sample by gender



Source: Author's research

The mean age (mean $\pm$ SD) of the subjects in the general group was 47.08 ± 12.94 years with a range between 20 and 67 years. The mean age of men did not differ statistically significantly from that of women (48.44 ± 12.29 years vs. 45.77 ± 13.61 years, $t = 1.131$; $P = 0.260$). According to IFCC guidelines (1987), the RefVAL version 4.11 program was used to analyze the data to determine reference ranges (Solberg, E., 2004). The IBM SPSS version 25 (2017) statistical program for social sciences was also used. The type of distribution of the anti-PLA2R1 results was checked. Representation overall figure 2.

Figure 2. Anti-PLA2R1 distribution pattern in men and women



Source: Author's research

The reference limits of anti-PLA2R1 obtained by us and group both sexes.

Table 4. And corresponding 90% confidence intervals general

	Total	Men	Women
Number of cases	120	59	61
Lower ref. limit (90% CI)	0.83 (0.39 – 1.33)	0.48 (0.44 – 1.34)	0.60 (0.16 – 1.28)
Upper ref. limit (90% CI)	17.83 (16.10 – 19.65)	18.88 (16.61 – 19.19)	18.81 (16.22 – 19.84)

Source: Author's research

To determine the critical value of anti-PLA2R1 that distinguishes patients from healthy individuals, we constructed a ROC (receiver-operating character). In the present study, the concentration of anti-PLA2R1 was also examined in the groups with PMN, SMN and ON. The results obtained were compared with those of the HC group. The concentration of anti-PLA2R1 in the four groups included in the study is presented as the mean and median, since we found high asymmetry (Skewness > 2.000) and non-Gaussian with the. Based on this, we

applied the Kruskal-Wallis test, which is based on the mean rank, and we found intergroup differences that the four groups in the study (table 5).

Table 5. Data on intergroup differences when comparing values

Group	Average value $\pm$ SD (RU/ml)	Median (RU/ml)	Average rank	Kruskal – Wallis (H, P)
PMN	213.97 $\pm$ 588.69	34.92	74.07	31.91, P < 0.001
SMN	6.34 $\pm$ 11.68	2.87	70.08	
ON	3.52 $\pm$ 3.91	2.08	40.32	
Controls	5.32 $\pm$ 3.91	4.82	82.09	

Source: Author's research

We found a statistically significant difference in the concentration.

4. DISCUSSIONS

The concept of “reference values” was first introduced in 1969 by Gräsbeck and Saris (1969). Our review of the scientific literature showed that there is currently no data on established reference ranges for the anti-PLA2R1 autoantibody index for the Bulgarian population. Most often, control groups are indicated in clinical studies in which the number of studied individuals varies. For example, in a clinical study conducted by Wang et al., a total of 478 patients were studied, including 30 healthy controls, 222 idiopathic MN (IMN), 29 SMN, and 197 other pathological types of nephropathy except MH, all of whom were Chinese (2023). In a study conducted by Ronchev (2025), 233 patients were included, divided into different groups: patients with PMN, SMN and other nephropathies, as well as healthy individuals (n = 120). In another clinical study conducted by Porcelli et al., 126 patients were diagnosed with IMN and 369 had other non-membranous nephropathies (2021). In a retrospective study conducted by Li et al., a total of 670 subjects including 374 IMN cases and 296 non-IMN (2022) were studied. The serum concentration of anti-PLA2R1 antibodies in all these studies was determined by the ELISA test used in our study. The individuals from the reference group that we used were selected without a statistically significant difference between the two sexes. For comparison, in a number of studies using a control group of healthy individuals, data on their distribution by sex are missing (Wang et al., 2023), and in others there is no data on their selection (Porcelli et al., 2021; Li et al., 2022). A key role in the diagnosis and therapy of patients with MN is the differentiation of primary from secondary forms (Huang et al., 2021). Clinical studies indicate a significant relationship between the concentration of serum anti-PLA2R1 antibodies and MN, as their expression is consistent with the results of a kidney biopsy (Wu et al., 2018), and their increased values have high specificity for diagnosing PMN. According to them, the change in serum anti-PLA2R1 antibody concentration is important for assessing the condition of patients, with low concentrations being associated with improvement in the condition, and their increase with the lack of remission or the presence of relapse. Other studies support this understanding of the need for testing serum anti-PLA2R1 autoantibodies for the diagnosis of PMN and its differentiation from SMN and ON (Liang et al., 2019; Ronchev, 2025). In the present study, we determined reference ranges for anti-PLA2R1 in clinically healthy individuals from the Bulgarian population. The results we obtained are: for the general group: 0.83 – 17.83 RU/ml; in the male group: 0.48 – 18.88 RU/ml and in the female group: 0.60 – 18.82 RU/ml. Data analysis showed that the lower reference range in the general group was slightly higher than that of men and women, and the upper reference range of the same group was slightly lower than the other two. Most often, the results of the study are presented as % (Wang et al., 2023) or quantitatively in RU/ml (Porcelli et al., 2021; Li et al., 2022). For the purposes of comparative studies, average values of the indicator were determined and used. For example, in a clinical study by Neto et al. (2020) in 19 patients with MN the mean value of anti-PLA2R1 was found to be 0.615 RU/ml with a confidence interval of 0.60 – 2.35 RU/ml. In a study by Dhaouadi et al., (2020) in 30 healthy individuals, the mean value was 2.0 RU/ml with a confidence interval of 2.0 – 2.3 RU/ml.

In the present study, we hypothesized that the concentration of anti-PLA2R1 antibodies in the serum of PMN patients is higher than that in the other groups and may improve the differential diagnosis between them. For this, we compared the serum concentrations in patients with PMN, SMN, ON with those of HC. We found a statistically significant difference between the PMN, SMN, ON groups in terms of the mean concentration of anti-PLA2R1, P < 0.0001. Due to the non-Gaussian distribution of the data, we compared the medians of the obtained results, which showed a statistically significantly higher median (4.82 RU/ml versus 34.92 RU/ml, P = 0.001) in PMN compared to HC and a lower median in ON (4.82 RU/ml versus 2.08 RU/ml, P = 0.002). We did not find a statistically significant difference in anti-PLA2R1 values in patients with SMN and HC (2.87 RU/ml versus 4.82 RU/ml on average, P = 0.187). Similar results to ours were obtained by Liu et al. (2018) in a comparative study between 119 patients with PMN (n = 57) and non-PMN (n = 62) and a control group of 22 HC. In a clinical study by Li et al. (2022) in patients with PMN (n = 113), SMN (n = 34) and HC (n = 53),

higher concentrations of anti-PLA2R1 were found in the PMN group (78.2 RU/ml) compared to SMN (1.85 RU/ml) and HC (9.02 RU/ml), $P < 0.05$.

Summarizing the results obtained, we can conclude that the reference limits of anti-PLA2R1 are a newly introduced indicator in clinical laboratory practice, which requires the use of our own reference limits.

5. CONCLUSIONS

The data published by us indicate that for the first time in Bulgaria, reference limits for anti-PLA2R1 in serum from clinically healthy individuals have been determined. The place and significance of this indicator for the diagnosis of PMN were also studied. In accordance with the understanding of statements published in some scientific studies, the serum concentration of anti-PLA2R1 antibodies can be used to distinguish PMN from SMN and ON.

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