INTRODUCTION

Glomerulonephritis belongs to various diseases and leads to chronic end-stage renal disease [1]. The history of membranous nephropathy dates back to 1946, when E. Bell first called this disease [2]. It belonged to a group of diseases kidney called Bright’s Disease Type II 9. One of the causes may be dysregulated functioning of the immune system, and thus the production of antibodies directed against its own antigens (autoimmune) or foreign antigens. Disorders in the activation and functioning of T and B lymphocytes are responsible for autoimmune processes. Their incorrect stimulation leads to the production of antibodies against own antigens. Predisposing factors for excessive activation of lymphocytes are: bacterial, viral infections and toxins. Activation occurs by: exposing antigenic epitopes to T lymphocytes or by the phenomenon of molecular mimicry, in which the antibodies cross-react with the antigen of the microorganism and host tissues. Mimicry is the results from homology between host tissues and microbial antigens. T lymphocyte activation, in turn, increases cytokine and lymphokine production.

Recent reports point to the huge role of regulatory T lymphocytes (CD 4 and CD25) in maintaining a balance between T lymphocyte activation and autoimmunity. An example here may be patients with Goodpasture's disease in whom the level of regulatory T lymphocytes is much lower than in healthy patients [3].

MN may also have genetic background relying on genetic variation within the HLA gene. The genetic conditioning of various forms of glomerulonephritis is not well understood. The presence of the HLA gene is not always indicative of the disease, and the corresponding HLA allele does not condition a given MN type. Although the HLA DR2 allele predisposes to Goodpasture's disease, patients without this allele may develop this disease. The HLA DR2 allele may be present in other diseases, e.g. narcolepsy. Other genes that have not yet been identified may also be involved in the development of the disease.

There are known several mechanisms of damage the glomeruli which leading to the development of inflammation. One of ways to damage the glomeruli is through a process involving circulating immune complexes (CICs), which are deposited in the glomeruli by binding to the Fc receptor on the surface of mesangial cells, directly in the mesangium or under the epithelium. Another way is to combine antibodies with internal glomerular antigens. In the next stage, the complement system is activated, which in turn activates a cascade of proteins, leading to the development of inflammation. The complement system can be activated in a classical manner, where C1q binds to the Fc receptor or by a lectin that has structural homology to the C1q protein. Stimulation of the complement system may also occur via an alternative route involving polysaccharide antigens, polymeric IgA or endotoxins. Soluble factors may also be involved in the development of inflammation. Not all have been known so far. The identified ones include: chemokines, cytokines, growth factors, vasoactive mediators, reactive oxygen species, proteases and proteins.

IMMUNODIAGNOSIS IN MEMBRANOUS NEPHROPATHY

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ABSTRACT

One of the diseases leading to chronic end-stage renal disease is membranous nephropathy (MN). The main cause of this disease is the formation of antibodies to foreign and native antigens. Membranous nephropathy can be conventionally divided into 2 types: primary form (when the primary disease is unknown) and secondary form. Detection of appropriate antibodies is one of the methods to recognize and differentiate primary and secondary forms. A large role in non-invasive diagnosis of MN and differentiation of the primary form from the secondary play antinuclear antibodies (ANA), antibodies against granulocyte cytoplasm (ANCA), antiglomerular basement antibodies (anti-GBM) and phospholipase A2 receptor antibodies (anti-PLA2R). Differentiation matters when choosing a treatment choice. In the primary form, it is immunosuppression, and in the form of secondary treatment, it consists in curing or controlling diseases that can cause symptoms of MN.

The aim: Analysis of serological methods helpful in immunodiagnosis of membranous nephropathy.

KEY WORDS: membranous nephropathy, immunodiagnosis, antibodies

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involved in the coagulation process. These factors activate inflammation cells in various ways [4].

Glomerulonephritis can be conventionally divided into 2 types: primary and secondary. The primary form of glomerulonephritis can be said when the underlying disease is unknown and the cause is related to the immune system or inflammation. The secondary form is part of another systemic disease (e.g. systemic lupus) [5]. 70-80% of patients develop primary MN, while about 20-30% develop secondary. Diagnosis of glomerulonephritis is based on the clinical picture and histopathological examination result from kidney biopsy. Unfortunately, the clinical picture does not always correlate with the histological picture. Hence, we distinguish clinical and histological types of MN.

Clinical types of glomerulonephritis:
1. In the form of changes in urine - low proteinuria / hematuria
2. In the form of macroscopic or recurrent haematuria
3. Nephritic syndrome - oliguria, edema, proteinuria <3g / 24h, hematuria
4. Nephrotic syndrome - proteinuria <3g / dl, hyperlipidemia, lipiduria and edema.
5. Rapid progressive glomerulonephritis - proteinuria, hematuria, renal failure within weeks, vasculitis
6. Chronic glomerulonephritis - proteinuria, hematuria, hypertension, chronic renal failure [6].

Histological types of glomerulonephritis:
1. proliferate
   • intra-capillary glomerulonephritis
   • extrapillary glomerulonephritis
   • mesangial glomerulonephritis
2. Membrane-proliferative glomerulonephritis
3. Membrane glomerulonephritis
4. Submicroscopic glomerulonephritis
5. Focal segmental glomerulosclerosis
6. Others

ANTII-NUCLEAR ANTIBODIES (ANA) IN LUPUS NEPHRITIS
Antinuclear antibodies (ANA) are a broad group of autoantibodies that are directed against components of the cell nucleus. They were first described by Coons and Kaplan in 1950 [7]. They occur in the course of many autoimmune diseases, mainly in rheumatic diseases. The incidence of antinuclear antibodies ranges from 20% to 100% [2]. In systemic lupus erythematosus (SLE), the highest incidence is achieved by anti-dsDNA (30-90%) and anti-nucleosomes (50-95%). SLE is a disease in which we observe clinical manifestations in various organs, including in the skin, mucous membranes, kidneys.

The most serious complication occurring in the course of SLE is lupus nephropathy - Lupus Nephritis [8-16]. It affects about 50% of patients with SLE. Despite many years of research, LN immunopathogenesis is not fully understood. It is generally accepted that anti-double-stranded DNA (anti-dsDNA) and anti-nucleosome antibodies. Anti-nucleosome antibodies are involved in the development of the disease, which appear in the early stages of the disease before anti-dsDNA antibodies and are correlated with disease activity. There is a relationship between high anti-dsDNA antibody titers and disease activity [17].

According to the latest EASI guidelines developed in March 2014. (The European Autoimmunity Standardization Initiative) ANA antibody diagnosis should be done in two stages:
1st STAGE - indirect immunofluorescence screening (IIFT) (Fig.1.)
2nd STAGE - confirmation of positive / borderline results by monospecific methods (ELISA, Immunoblot)

ANTI-NEUTROPHIL CYTOPLASM ANTIBODIES (ANCA) IN VESSELS INFLAMMATIONS
If the MN secondary form is observed in the course of vasculitis, an important element of diagnostics is the assessment of antibodies against neutrophil cytoplasm (ANCA). The recommended substrate for ANCA antibodies evaluation is ethanol-fixed human neutrophils. This way of antigen fixation allows the assessment of two basic types of fluorescence:
• cANCA (cytoplasmic ANCA) (Fig. 2) for which antibodies against proteinase 3 (PR3) are most often responsible. The cANCA group also includes the less common anti-BPI (Bactericidal permeability increasing protein), anti–CAP57 and very rarely anti- myeloperoxidase (MPO) antibodies. The frequency of cANCA antibodies is (Tab. 1) [20, 21]:
• pANCA (perinuclear ANCA) (Fig 3), represents a much larger group of autoantibodies. The most important are anti-myeloperoxidase (anti-MPO) antibodies. In addition, the following antibodies can also be included: anti-lactoferrin, anti- elastase, anti-BPI, anti-cathepsin G, anti-lysozyme and anti-β–Gluconidase [21-23]. Antibodies to pANCA occur in such diseases as (Tab. 2) [20, 25, 26].

A separate group of ANCA antibodies are so-called DNA-ANCA (formerly pANCA sensitive to formalin). They occur most often in the course of ulcerative colitis, the incidence in UC is 67%. The main target antigen is lactoferrin [20, 27]. Recommendations from 1999 recommend a two-steps ANCA antibody evaluation strategy (International Consensus Statement on Testing and Reporting ANCA) (Fig: 4):

Optimal diagnostics in the case of negative results (confirmation by a mono-specific ELISA test) is aimed at detecting antibodies in patients whose antibody levels may have fallen after treatment or increased during remission. Minimal diagnostics allow the release of a negative result based only on IIFT [28].

ANTI-GLOMERULAR BASEMENT ANTIBODIES (GBM) IN GOODPASTURE’S SYNDROME
Anti-GBM antibodies occur in the course of Goodpasture's disease by many also called Anti-GMB disease.
According to the latest classification, it is included in the group of diseases associated with inflammation of small vessels. The target antigen for anti-GBM antibodies is the non-collagen NC domain of the 3 alpha chain collagen type IV (alpha3 (IV) NC1) located in the basement membrane of the glomeruli. Clinical manifestations in Goodpasture’s disease are associated with glomeruli where inflammation usually results in severe, rapidly progressive inflammation. Glomeruli and with respiratory symptoms. When modern treatment induced, renal function is preserved in less than 1/3 of patients during 6 months of follow-up. Cui Z et al. proved that anti-GBM antibodies can also appear in the serum of healthy patients in lower titers. Anti-GBM antibodies have a high prognostic value, and can
detect anti-PLA2R antibodies in patients' serum by two methods: thus damage to podocytes and glomeruli (proteinuria). We can complement, overproduction of laminin and collagen IV, and extracellular and intracellular. Anti-PLA2R antibodies activate of podocytes in human glomeruli and consist of 2 domains: receptor. To date, two groups of PLA2 receptor (M and N) have been phospholipaseA2 (transmembrane glycoprotein) anti-PLA2R re-

Antibodies to pANCA occur in such diseases as:

<table>
<thead>
<tr>
<th>Prevalence</th>
<th>Microscopic vasculitis (MPA)</th>
<th>42-70%</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Churg-Strauss Syndrom(CSS)</td>
<td>18-60%</td>
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<tr>
<td></td>
<td>Inflammatory bowel's diseases</td>
<td>67%</td>
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<tr>
<td></td>
<td>Primary sclerosing cholangitis</td>
<td>?</td>
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<td></td>
<td>Autoimmune liver diseases</td>
<td>?</td>
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<tr>
<td></td>
<td>Collagenosis (SLE)</td>
<td>9-25%</td>
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<td></td>
<td>Rheumatoid Arthritis</td>
<td>3-20%</td>
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<td></td>
<td>cancers</td>
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<td>infections</td>
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be detected by IIFT (Fig. 5), ELISA and Immunoblot [29].

**DISCUSSION**

Differentiating the primary and secondary forms of MN is an important element when choosing the right treatment. Treatment of glomerulonephritis is multidirectional. Its purpose is to stop the progression of kidney damage. In the primary form, immunosuppression is most often used, while in the secondary form, cure or optimal control of the disease that causes membranous MN is crucial. The clinical and histopathological picture of kidney biopsy plays a huge role in the diagnosis of glomerulonephritis. The clinical picture does not always correlate with the histological picture. Therefore, MN can be divided into clinical and histological types of MN. The same clinical picture may have different etiologies, and the same cause of the disease may give a different histological picture. Therefore, the basis for the diagnosis of glomerulonephritis is a kidney biopsy and observation of kidney tissue under a light, fluorescent or electron microscope. The characteristic picture of changes allows to diagnose the disease [5].
Recent studies show the huge role of antibodies involved in disease development that are useful in the diagnosis and differentiation of membranous nephropathy. The ability to detect the level of antibodies can facilitate both the diagnosis and the distinction of primary and secondary forms without invasive methods for diagnosing MN.

Autoantibody determination is useful in monitoring disease activity and treatment efficacy. The most important parameters of the examined include antinuclear antibodies (ANA), anti-neutrophil cytoplasmic antibodies (ANCA), anti-glomerular basement antibodies (anti-GBM) and phospholipase A2 receptor antibodies (anti-PLA2R). In 2009, Beck and colleagues found PLA2R antibodies that play an important role both in the diagnosis of membranous nephropathy and differentiation because they were detected in 70% of patients suffering from primary rather than secondary [31]. Gunnarsson and colleagues, on the other hand, have proven that PLA2R antibodies are absent in patients with membranous lupus nephropathy, which is a secondary disease in the presence of SLE [35]. The secondary form of membranous nephropathy is also characterized in many cases by the presence of ANCA antibodies associated with vasculitis [20]. Anti-DNA antibodies and anti-nucleosomes play a significant role here, as they correlate strongly with disease activity. Cui Z et al. Claim that anti-GBM antibodies have a high prognostic value and may also appear in the serum of healthy patients in lower titers [27].

CONCLUSION

The above work focuses on serological diagnostics, which is helpful in diagnosing, differentiating as well as monitoring the treatment and estimation of relapses after kidney transplantation in diseases related to glomerulonephritis. In the case of suspected Lupus nephrits, testing for the presence of antinuclear antibodies (ANA), in particular anti-dsDNA, and anti-nucleosomes is helpful. As for diseases associated with vasculitis in glomerulonephritis, we detect ANCA antibodies. Goodpasture's syndrome is characterized by the presence of Anti-GBM. Novelty are anti-PLA2R antibodies whose incidence in primary membranous nephropathy is 100%.

REFERENCES