

Review

Membranous Glomerulonephritis: Overview of the Role of Serum and Urine Biomarkers in the Management

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Abstract: Detection of PLA₂R and THSD7A among primary membranous glomerulonephritis (MGN) patients transformed the diagnosis, treatment monitoring and prognosis. Anti-PLA₂R can be detected in 70-90% of primary MGN patients while anti-THSD7A in 2-3% of anti-PLA₂R negative primary MGN patients depending on the technique used. Serum and urine samples are less invasive and non-invasive respectively and can detect the presence of anti-PLA₂R and anti-THSD7A with higher sensitivity and specificity, significant in patients' monitoring and prognosis better than exposing patients to frequent biopsy which is an invasive procedure. *Different techniques of detection of PLA₂R and THSD7A in patients' urine and sera were reviewed with the aim of providing newer and alternative techniques.* We proposed the use of biomarkers (PLA₂R and THSD7A) in making the diagnosis, treatment decision and follow up of patients with primary MGN. We also reviewed other prognostic renal biomarkers like retinol binding protein(RBP) and beta-2 microglobulin in order to detect progression of renal damage for early intervention.

Keywords: M-type phospholipase A2; Thrombospondin type containing domain A7; Retinol-binding protein; Beta-2 microglobulin; membranous glomerulonephritis; neutral endopeptidase

1. Introduction

Membranous glomerulonephritis (MGN) is characterized by the deposit of immune complex at the subepithelial region of glomerular membrane and formation of perpendicular projections similar to the glomerular basement membrane (GBM) found between podocyte cytoplasm and GBM(Glasscock, 2010; Larsen, *et al.*, 2012). The histological characteristics of MGN include GBM thickening, staining of the granule of immunoglobulin G subtype 4(IgG4), complement C3 staining along the periphery of the capillaries of glomerulus and deposition of the immune complex at the subepithelium(Fogo AB, *et al.*, 2011).

Studies have shown that MGN being the commonest cause of nephrotic syndrome among adults, consist of up to 20% of cases among Hispanic and African Americans. White people are most affected, followed by Asians, Africans and Hispanics(Salant DJ, 2015; Cattran DC, 2017). MGN account for 1.5-9% of the nephrotic syndrome among children and 21-35% in adults. The disease is predominantly seen among adults aged 40-50 years of birth with a ratio of male to female 2-3:1(Cattran & Brenchley, 2017).

Idiopathic or primary MGN is the commonest type seen in about 75% of patients with MGN while the remaining 25% manifest as a disease secondary to other conditions, mainly infection (Lefaucheur *et al.*, 2006; Zeng *et al.*, 2008; Seitz-Polski *et al.*, 2016; Feng *et al.*, 2016).

It is very difficult to differentiate primary and secondary MGN based on their clinical presentations and laboratory evaluations. Therefore, a good leading history, clinical and laboratory findings (including renal biopsy) are crucial in differentiating the two types of MGN (KDIGO, 2012). Diagnosis of MGN is traditionally made through electron microscopy, light microscopy and immunofluorescence techniques from renal biopsy (Mastroianni-Kirsztajn, *et al.*, 2015).

In this review, we aim at highlighting the newer techniques used in making a diagnosis of MGN by detecting biomarkers in serum and urine samples of MGN and the importance of those biomarkers in patients' management.

2. Material and method

Published articles and thesis on various methods used in the detection of anti-PLA₂R, THSD7A, RBP and B2-microglobulin in MGN patients were searched using Pubmed, Springer, Google Scholar and Science Direct.

2.1. The biomarkers

Neutral endopeptidase (NEP) is the first human biomarker for MGN identified in early 2000, seen in patients with alloimmune neonatal MGN. This involves vertical transmission from mother to her offspring following sensitization during a previous pregnancy (Hu, Xuan, Hu, Lu, & Qin, 2013). Therefore, it is very important to screen families with a history of membranous nephropathy as part of their antenatal care (Debiec H, *et al.*, 2004; Hu, *et al.*, 2013).

Beck *et al.*, in 2009 conducted a breakthrough study where M-type phospholipase A2 receptor (PLA₂R) was detected as a biomarker for MGN using western blot technique. More recently, another biomarker, thrombospondin domain containing 7A (THSD7A) was discovered to complement PLA₂R. This biomarker is detected in sero-negative anti-PLA₂R primary MGN patients except for some rare conditions where anti-PLA₂R and anti-THSD7A can be detected (Larsen, *et al.*, 2015). Anti-THSD7A cannot be detected in normal individual or patients with secondary MGN (Prunotto M, *et al.*, 2010; Hofstra, *et al.*, 2011).

Anti-THSD7A occur in 2.5- 5% of patients with idiopathic MGN and does not appear in secondary MGN. Except for few where both PLA₂R and THSD7A were found positive, THSD7A is only detected among those MGN patients who are PLA₂R negative (Gosink, 2016; L. Y. Liu *et al.*, 2016).

2.1. Pathogenesis

Immune complex formation in the subepithelial surface of glomerular complex membrane is central to the formation of membranous glomerulonephritis (Ling *et al.*, 2015). Three major mechanisms had been proposed so far (Ling *et al.*, 2015).

The first hypothesis laid more emphasis on entrapment of preformed immune complexes passively which was resulted from higher intra-glomerular pressure and negatively charged capillary which force the protein across the glomerular capillary wall. An example is in lupus nephritis (Shlomchik & Madaio, 2003; Wilcox & Hirshkowitz, 2015).

In the case of second hypothesis, there is localization of circulating antigens in the subepithelial aspect of the glomerular basement membrane, so the antigen form in-situ immune complex deposit with the antibodies. This is seen in hepatitis B (Bhimma & Coovadia, 2004; Ju-Young Moon, 2012) hepatitis C (Sandri, Elewa, *et al.*, 2011; Morales, *et al.*, 2012) Helicobacter pylori (Dede *et al.*, 2015) patients diagnosed of MGN (Beck, L. H., & Salant, 2010; Lien YH, 2011).

Third mechanism focus on the binding of autoantibodies to antigens to podocyte membrane, leading to immune complex deposition. A lot of studies done to describe the pathogenesis of MGN, ranging from the Heymann rat model of 1959 where membranous glomerulonephritis was induced

using Lewis rats with crude kidney extract (Heymann W, *et al.*, 1959). The use of megalin in 1980s by Kerjaschki and Farquhar where they show that oxygen radicals responsible for glomerular

damage leading to proteinuria (Farquhar MG, *et al.*, 1995). No anti-Megalina antibody was recorded to have been found in patients with MGN, therefore megalin cannot be associated with proteinuria in human (Ling *et al.*, 2015; Ronco & Debiec, 2015).

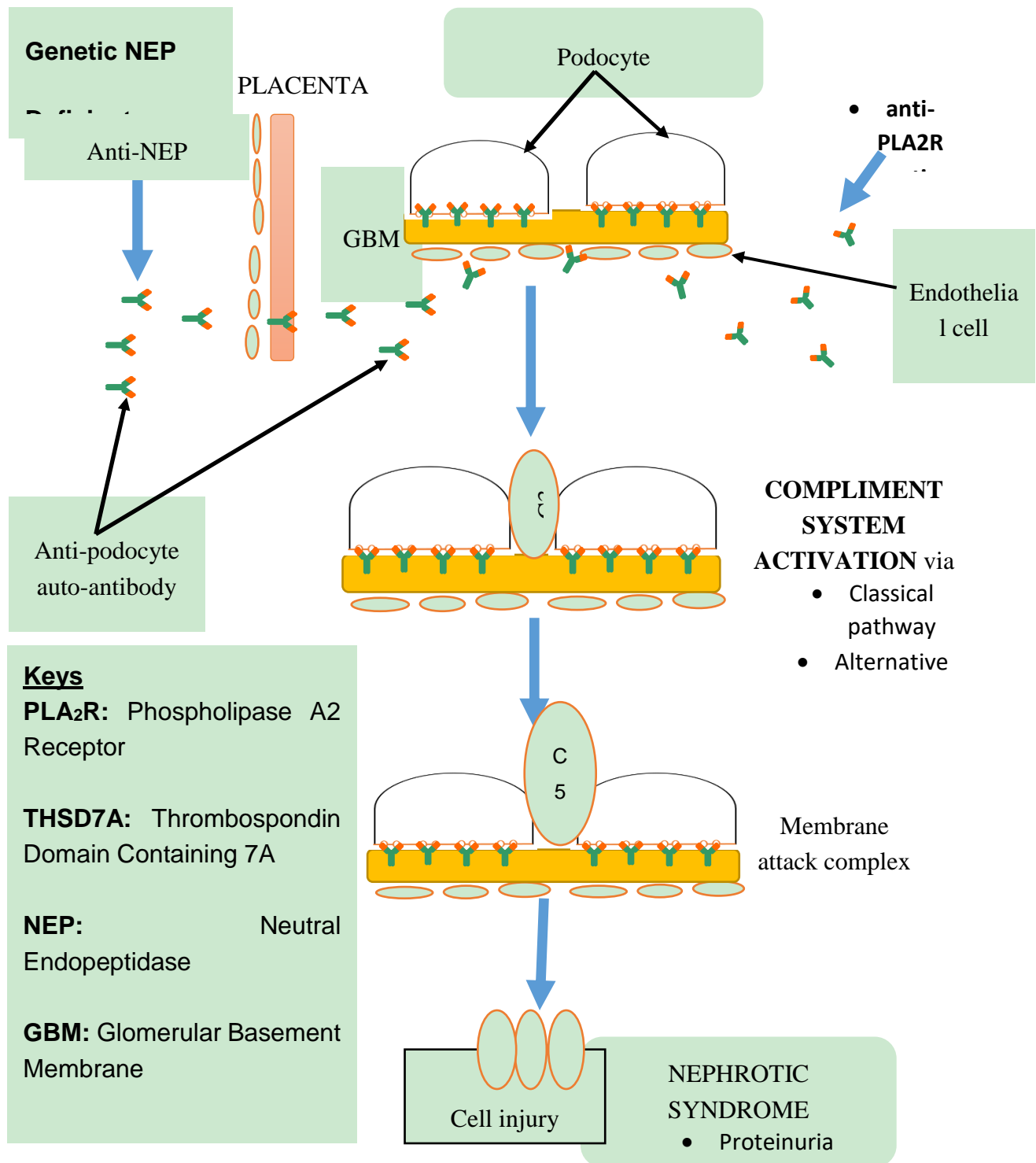


Figure . Mechanism of anti-podocyte (anti-NEP, anti-PLA₂R, anti-THSD7A) antibody-mediated disease in membranous glomerulonephritis, showing the role of complement system in the pathology.

1. Methods of detecting biomarkers

The following methods can be used in detecting PLA₂R and THSD7A antibodies in patients' sera. Enzyme-Linked Immunosorbent Assay (ELISA) method, Laser Bead Immunoassay (ALBIA), Luciferase immunoprecipitation system (LIPS)(Burbelo & Beck Jr, 2017). Recently, different methods of detecting anti-PLA₂R and THSD7A are available commercially due to rise in their clinical applications in patients' management.

1.1. Western blot technique

Western blot technique is the first method used to detect the expression of anti-PLA₂R in patient's serum with primary MGN(Beck LH Jr, *et al.*, 2009). In western blot technique, proteins are separated based on their molecular weight through gel electrophoresis(Tahrin Mahmood, 2012). The technique was first reported to have a sensitivity of about 70% with up to 96% specificity(Beck LH Jr, *et al.*, 2009). There were also reports on an improved variant of the Western blot technique(specially designed to detect very low anti-PLA₂R) with sensitivity even greater than 90%(Qin W, *et al.*, 2011). However, the method is only suitable for smaller sample size and also requires expertise(Dahrnich C, *et al.*, 2013).

1.1. Recombinant cell-Indirect Immunoassay (RC-IFA)

In this case, the substrate is human cell-line HEK293-overexpressing the full-length PLA₂R₁(Hoxha E, *et al.*, 2010). RC-IFA can be used to make a diagnosis and monitor primary MGN patients. It was shown to have sensitivity of 75% and specificity of near 100%(Hofstra JM, *et al.*, 2012). RC-IFA has been considered as the most suitable method for the detection of anti-PLA₂R₁ (Debiec H, 2011). It can also be used as a reference technique to determine several antibodies against the membrane proteins of the outer surface like NMDR in autoimmune encephalitis(Wandinger KP, *et al.*, 2011) aquaporin 4 in neuromyelitis optica (Waters PJ, *et al.*, 2012), and Crohn's disease(Komorowski L, *et al.*, 2012).

Despite all the above advantages, it has its own limitations, RC-IFA requires technical know-how and special equipment like fluorescence microscope, it is liable to subjective interpretation and it can only give a semi-quantitative variable.

1.1. Time-resolved fluoroimmunoassay(TRFIA)

This is a highly specific antigen-antibody binding reaction by measuring light emission from labels conjugated from protein (Soini, E and Hemmila, 1979; Hemmilä, *et al.*, 1984) This assay has high sensitivity, used in the quantitative measurement of serum PLA₂R-Ab immunoglobulin(IgG4)(Zhang *et al.*, 2017).

1.1. Laser Bead Immunoassay (ALBIA)

Behnert *et al.*, in 2013 reported the use of Laser Bead Immunoassay (ALBIA) using an in vivo expressed recombinant human PLA₂R in order to take care of limitations of RC-IFA (quantitative assay, high objectivity in assessment) (A. Behnert, 2013). Further study was carried out to compare with two commercially available immunoassays. It was proved that ALBIA correlate better with RC-IFA than with ELISA($P = 0.003$) and the overall result showed sensitivity and specificity of 60% /98.6%, and 56.2% / 100% for ALBIA and RC-IFA respectively(Behnert *et al.*, 2014). Despite this remarkable result for ALBIA, it is not available commercially.

1.1. Luciferase Immunoprecipitation System (LIPS)

Another technique, LIPS assay that makes use of light emitting proteins. This can detect different types of antibodies including anti-PLA₂R(Burbelo, P.D., *et al.*, 2015). The PLA₂R LIPS assay is quantitative and highly sensitive. It has a sensitivity of near 100% and specificity of 100% better than most of these methods of detecting PLA₂R. It can also positively correlate with proteinuria and

disease process ($p < 0.005$) (Burbelo, & Beck Jr, 2017). More studies needed to prove the above claim and its use are limited to research only (not yet available commercially).

1.1. Enzyme-Linked Immunosorbent Assay (ELISA)

Therefore, there is an urgent need to develop a standardized ELISA to overcome these shortcomings and to give identical diagnostic accuracy for better clinical importance. This involves the expression of PLA₂R1 in HEK293. This technique was used to analyse sera from 200 primary MGN patients, 27 secondary MGN and 291 healthy individuals. The results indicated a remarkable sensitivity of 96.5% and specificity of 99.99%. The result has correlated significantly well with clinical findings of patients and the results obtained from RC-IFA (Dährnich *et al.*, 2013).

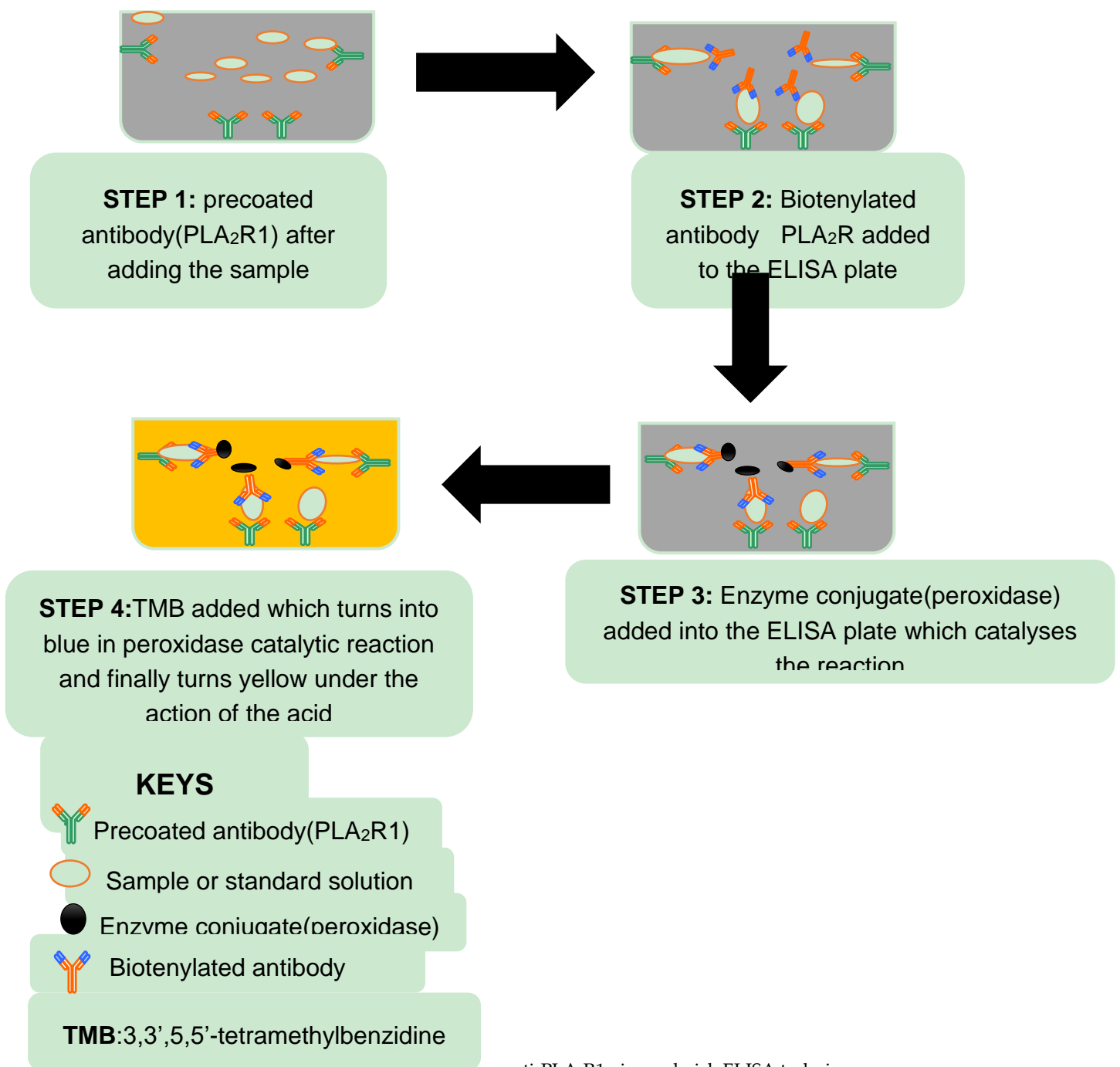


Figure 2. Mechanism of detecting anti-PLA₂R1 via sandwich ELISA technique.

Timmermans *et al.*, conducted a comparative study involving different methods of detecting PLA₂R antibody among 158 patients of which 142 were primary and 16 were secondary MGN. Western blot, ELISA and IIFT techniques were compared and the results showed the specificity of $\geq 97\%$ for all techniques and a sensitivity of 68% for IIFT and 72% for both ELISA and western blot techniques. ELISA technique may be the preferred method because it can be used for large sample size, both qualitative and quantitative measurement. It is less time consuming, requires less technical know-how and can be interpreted objectively. This clearly showed the superiority of the ELISA method in terms of commercial availability and clinical application (Ayalon, Jr, & Schlumberger, 2014).

| Techniques | No. of subjects | Sensitivity (%) | Specificity (%) | References |
|--------------|-----------------|-----------------|-----------------|-------------------------------------|
| Western blot | 37 | 70 | 96 | (Beck LH Jr, <i>et al.</i> , 2009) |
| ALBIA | 157 | 60 | 96 | (Behnert <i>et al.</i> , 2014) |
| LIPS | 45 | 97 | 100 | (Burbelo, & Beck Jr, 2017) |
| ELISA | 200 | 96.5 | 99.99 | (Dähnrich <i>et al.</i> , 2013) |
| TRFIA | 39 | 89.7 | 100 | (Zhang <i>et al.</i> , 2017) |
| RC-IFA | 75 | 75 | 100 | (Hofstra JM, <i>et al.</i> , 2012). |

Table 1. showing various techniques used in detecting PLA₂R.

2.3. Testing for anti-PLA₂R and anti-THSD7A in serum

A meta-analysis involving 19 studies and 1160 patients were conducted to investigate the clinical importance and the accuracy of histological and serological PLA₂R in differentiating primary and secondary membranous glomerulonephritis (MGN). The overall results showed a sensitivity of 0.68%, specificity of 0.97% and diagnostic odds ratio (DOR) was 3.75 while the area under the receiver operating curve (AUROC) was 0.82 for serum anti-PLA₂R with a substantial heterogeneity ($I^2 = 86.42\%$). In the case of PLA₂R staining, the overall sensitivity was 0.78%, specificity was 0.91%, DOR(34.70) and AUROC (0.84) without heterogeneity ($I^2 = 0\%$). Thus, serological anti-PLA₂R can be of good diagnostic value, but patients' clinical presentation and PLA₂R staining must be considered in case of seronegative patients (Dai, *et al.*, 2015; Weiyang Li, 2018).

In another study among the Chinese patients involving 57 biopsies proven idiopathic MGN, 62 non-MGN and 22 healthy individuals. ELISA technique was used to quantify anti-PLA₂R in the serum. The results obtained are shown by the table below.

| Cut-off value (RU/ml) | Sensitivity (%) | Specificity (%) | Positive predictive value(%) | Negative predictive value(%) |
|-----------------------|-----------------|-----------------|------------------------------|------------------------------|
| 2.0 | 75 | 82.5 | 69.1 | 86.3 |
| 2.6 | 78.9 | 91.7 | 86.5 | 86.5 |
| 14 | 59.6 | 95.2 | 89.5 | 77.7 |
| 20 | 50.9 | 96.4 | 90.6 | 74.3 |
| 40 | 47.4 | 97.6 | 93.1 | 73.2 |

Table 2. showing sensitivity, specificity, positive predictive value and negative predictive value using different cut-off points.

The above study recommended 2.6RU/ml to make a diagnosis(Y. Liu *et al.*, 2018).

Thrombospondin domain containing 7A(THSD7A) is a form of membrane-associated N-glycoprotein found within endothelial cells of the human umbilical vein and also expressed in blood vesicles of the placenta(Wang *et al.*, 2010).

A study conducted in 154 primary MGN patients, demonstrated that 15 of the 154 patients have antibodies for only anti-THSD7A(Tomas NM, 2014). Subsequently, more researches were conducted to detect the level of anti-THSD7A in the serum of MGN patients. Anti-THSD7A can be detected in serum and tissue of primary MGN patients with THSD7A(Sharma SG, 2017). Unlike PLA₂R, THSD7A does not show any significant preference for serum creatinine, albumin and proteinuria levels(Iwakura T, *et al.*, 2015). No significant correlation was found between THSD7A and proteinuria(Hoxha E, *et al.*, 2016).

In a meta-analysis of 10 different studies involving 4121 patients with primary MGN and based on sample size, race and detection method, it was found that anti-THSD7A was 3% in all patients and 10% among those without anti-PLA₂R. thus, this clearly showed a clear difference in the prevalence of anti-THSD7A based on the sample size but not many differences were observed among races(Ren *et al.*, 2018).

Serum analysis using indirect immunofluorescence was conducted among 31 THSD7A positive stained patients from the 1318 biopsy-proven MGN. The results showed a strong correlation between serum and tissue stain for MGN in all patients($p < 0.001$)(Sharma & Larsen, 2018).

2.4. Testing for anti-PLA₂R and anti-THSD7A in urine

Apart from serum, it was also found that anti-PLA₂R can be detected in the urine of a primary MGN patient. A urine sample is non-invasive and can detect renal damage more than serum, therefore it is important to demonstrate whether anti-PLA₂R can be detected in urine. To do this, a study was conducted on 28 primary MGN and 12 secondary MGN patients in China using ELISA and IIFT. The result showed that 18 of the 28(64.3%) primary MGN patients tested positive for IIFT serum PLA₂R while 19 of the 28 (67.9%) had IIFT positive urinary anti-PLA₂R. The antibody titre of anti-PLA₂R from primary MGN patients in urine and serum is higher than the corresponding titres from secondary MGN ($p < 0.05$). Statistical analysis indicated a positive correlation between urinary anti-PLA₂R and serum anti-PLA₂R. More studies needed to prove that anti-PLA₂R can be detected an insignificant amount in the urine of primary MGN patient(Yu Wang, *et al.*, 2018).

Despite several studies involved in the detection of THSD7A in tissue and serum, no known published study regarding its detection in patients' urine.

2.5. Clinical feature

Nephrotic range proteinuria is the commonest presentation among MGN patients. It occurs in 70-80% of patients associated with oedema, hypoalbuminaemia and hyperlipidaemia while the remaining patients present with subnephrotic range proteinuria(Ponticelli C, Passerini P, 2006; Kemp, W. L., *et al.*, 2008; Barbour S, Reich H, 2013; Thompson A, *et al.*, 2015 ; Salant DJ, 2015; Cattran DC, 2017). The renal function may be normal or slightly impaired at diagnosis. An abrupt change in renal functions may call for a prompt investigation to look for possible superimposed conditions like bilateral renal thrombosis, drug toxicity and crescentic glomerulonephritis(Nachman PH, Jennette JC, 2012). Other features include haematuria, hypertension is mostly not specific to idiopathic membranous nephropathy(Yahya, *et al.*, 2012).

A study involved the administration of THSD7A-specific IgG to mice thereby leading to massive proteinuria and histomorphological changes of MGN. The above findings showed that anti-THSD7A antibodies might interfere with the integrity of podocyte resulting in damage of cells and subsequently proteinuria(Ren *et al.*, 2018).

Most of the patients presenting with subnephrotic proteinuria are asymptomatic and have a natural history different from those with nephrotic range proteinuria. About 40% will have spontaneous remission, needing just conservative management while the remaining 60% may develop nephrotic range proteinuria within 2 years of presentation especially when the anti-PLA₂R antibody is still present (Stanescu HC, *et al.*, 2011; Guerry MJ, *et al.*, 2016). The progression of the disease is four times accelerated which become synonymous to those that presented with nephrotic syndrome *ab initio* (Hladunewich MA, *et al.*, 2009). This is another scenario in which anti-PLA₂R measurement may be important (Cattran & Brenchley, 2017).

2.6. Diagnosis

Previous studies showed that anti-PLA₂R is now an established parameter for diagnosing primary MGN, differentiating it from secondary type, monitoring treatment and prognosis (Pourcine *et al.*, 2017). The antibody titre helps in monitoring treatment

more than proteinuria as the change in titre is immunological, so it precedes the change in proteinuria (Mastroianni-Kirsztajn, *et al.*, 2015).

All patients with biopsy-proven MGN should be screened for anti-PLA₂R/THSD7A as well as hepatitis C, hepatitis B, lupus nephritis antigens and malignancies to rule out secondary causes (Francis JM, Beck LH Jr., 2011; Debiec H, 2016; De Vriese, *et al.*, 2017).

Most ELISA authors define positivity of anti-PLA₂R using a cut-off point of 20 RU/mL, some use 14 RU/mL, 2.6 RU/mL or 2RU/mL as their cut-off point value to define positivity (Montinaro, 2018; Qu *et al.*, 2018; Yang, *et al.*, 2018; Bobart *et al.*, 2019). In some cases, the cut-off point value is obtained by measuring the anti-PLA₂R of apparently normal subjects without any renal compromised (Jullien *et al.*, 2017).

2.7. Prognosis

Recent studies conducted within 5 years have shown that anti-PLA₂R concentration are correlated with urinary protein and disease activities; the antibodies are usually undetectable in spontaneous or drug induced remission patients and reappear when there is relapse (Yang SH, *et al.*, 2013; Zhou X, *et al.*, 2013; Hofstra JM, *et al.*, 2014; Hoxha E, *et al.*, 2014; Hoxha E, *et al.*, 2014).

Toronto risk score has been used to predict the prognosis of MGN patients. It is calculated based on creatinine clearance at diagnosis, highest sustained 6 months period of proteinuria and slope of creatinine clearance over 6 months. It has an accuracy level of up to 90%. However, there are challenges associated with this method which include complex calculation, prolonged observation of up to 18 months which may delay treatment. Recently, biomarkers like PLA₂R, retinol binding protein (RBP), beta-2 microglobulin can be used to predict prognosis among patients with MGN (Pei Y, Cattran D, 1992; Cattran DC, *et al.*, 1997). A retrospective study involving 33 non-nephrotic MGN patients showed that those with anti-PLA₂R positive (48%) were more at risk of progressing to nephrotic syndrome compared to those anti-PLA₂R negative patients. In addition, patients with high anti-PLA₂R titre are more at risk of adverse effects of immunosuppressive drugs and end-stage renal disease compared to those with no anti-PLA₂R by the end of follow-up. More studies involving a large number of patients are needed to confirm the above claim as the small number of patients in this study and follow up duration which is too short rendered it hard to categorically determine the outcome (Hoxha *et al.*, 2014).

In order to accurately predict the progression of MGN, watchful waiting method was adopted, this involves 24 hours urinary protein and creatinine clearance monitoring for at least 6 months and comparing the result with nephrotic range proteinuria (Cattran DC, *et al.*, 1997).

Important to know that the presenting proteinuria is inversely proportional to the rate of spontaneous remission (van den Brand JA, *et al.*, 2014). It was observed that there is a high chance of spontaneous remission if a 50% reduction in proteinuria is achieved within the first year irrespective of the initial level of proteinuria. About 32% can undergo spontaneous remission within 14 months and up to 62% in 5 years especially among MGN patients with decreased (low) anti-PLA₂R and anti-THSD7A (Polanco N, *et al.*, 2010; Hofstra *et al.*, 2011).

More recent studies support a watchful waiting approach and also indicated that spontaneous remission can occur even when the presenting proteinuria is greater than 12g/day (Polanco N, *et al.*, 2010).

Further studies involving natural history and spontaneous remission in south-east Asia and Malaysia are needed.

2.8. Retinol Binding Protein

This is a low molecular weight protein (LMW) synthesized in the liver, found in circulation bind to transthyretin. RBP is not filtrated by the glomerulus due to complex formation with transthyretin. But 4-5% of RBP circulates freely, filtered by glomerular basement and then reabsorbed at the proximal convoluted tubule (PCT). For the above fact, urinary RBP is an important biomarker of PCT dysfunction. The high value of RBP is associated with poor prognosis (high chance of patient tilting towards chronic kidney disease). Recently, it was shown that urinary RBP was associated with risks of renal replacement therapy and a rise in serum creatinine among diabetic patients (Domingos MAM, 2016). Patients with average or low urinary RBP are more likely to achieve remission earlier (Lin *et al.*, 2016).

Beta-2 Microglobulin

β 2-microglobulin can predict the prognosis of MGN (Branten AJ, *et al.*, 2005; van den Brand JA, *et al.*, 2014) β 2-microglobulin has 88% sensitivity and 91% specificity in determining the prognosis in renal failure with the threshold level at 40 mg/min (Branten AJ, *et al.*, 2005). However, when re-evaluated, both biomarkers show low sensitivity and specificity compared to the initial result. This may be due to conservative therapy (van den Brand JA, *et al.*, 2014). There are no significant differences between prognostic accuracies from β 2-microglobulin and Toronto Risk Score (van den Brand JA, Hofstra JM, 2012).

1. Treatment of idiopathic MGN

Serum and urine biomarkers (PLA₂R and THSD7A) are now used in monitoring the efficiency of immunosuppressive therapy. The biomarkers can also be used to compare two immunosuppressive drugs by measuring the serum level of PLA₂R and THSD7A before, during and after treatment (Hladunewich MA, *et al.*, 2014) (Beck *et al.*, 2011; Ruggenenti *et al.*, 2015; Dahan *et al.*, 2017). Rituximab can be used to reduce PLA₂R. However, the total dose needed to clear anti-PLA₂R still remains unclear and may be patient dependent (Dahan, *et al.*, 2018).

1. Potential MGN therapies

Another suggestion was the use of drugs that inhibit the factors that activate autoreactive B cells. In this case, Belimumab, act by reducing the production of autoantibodies by binding to BlyS (Stohl W, *et al.*, 2012). In a study involving 14 patients, it was found that Belimumab caused significant reduction in anti-PLA₂R, proteinuria and normalized serum albumin level (Willcocks L, *et al.*, 2015).

3. Conclusion

Standardized ELISA method is the best so far considering its ability to measure both qualitative and quantitative variable, less time required, easier to perform, high sensitivity and specificity and also readily available and affordable commercially.

Serum or urine samples should be used to determine the level of anti-PLA₂R/ anti-THSD7A in order to make diagnosis, monitor patients' treatment and in order to determine the prognosis especially among patients who can not withstand renal biopsy. Serum and urine samples can determines when to commence or stop immunosuppressive therapy.

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