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RETRACTED: Role of Serum and Urine Biomarkers (PLA_2R and THSD7A) in Diagnosis, Monitoring and Prognostication of Primary Membranous Glomerulonephritis

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Abstract: Differentiating primary and secondary membranous glomerulonephritis (MGN) using biomarkers for MGN is essential in patients' diagnosis, treatment and follow-up. Although biopsy has been the primary tool in making the diagnosis, not all patients can withstand it due to its invasive nature, and it cannot be used to monitor treatment. Hence, there is the need for less invasive or even non-invasive biomarkers for effective diagnosis, treatment monitoring and prognostication. This study aimed at providing an alternative way of differentiating primary and secondary MGN using enzyme-linked immunosorbent assay (ELISA) technique for serum and urine biomarkers (M-type phospholipase A2 receptor (PLA_2R) and thrombospondin type-1 domain-containing 7A (THSD7A)) for prompt diagnosis, treatment and prognosis. A total of 125 subjects, including 81 primary and 44 secondary MGN subjects, were diagnosed from January 2012 to October 2019 at Hospital Serdang and Hospital Kuala Lumpur from which 69 subjects consisting of 47 primary and 22 secondary MGN subjects participated in the study. Of these, 13 primary MGN subjects were positive for both serum and urine anti-PLA_2R antibodies (Ab) whereas only one secondary MGN subject associated with hepatitis B virus was positive for both serum and urine anti-PLA_2R Ab. At the same time, anti-THSD7A Ab was found positive in four primary MGN subjects and two secondary MGN subjects with malignancy.

Keywords: membranous glomerulonephritis (MGN); M-type phospholipase A2 receptor (PLA_2R); thrombospondin domain-containing protein 7A (THSD7A); prognostication

1. Introduction

Primary membranous glomerulonephritis (MGN) is one of the most common types of primary glomerulonephritis among adults [1,2], associated with a frequent increase in prevalence in Southeast Asia [3] and Malaysia [4] in particular. Although biopsy remains the confirmatory test for MGN, the clinical outcome is variable and often unpredictable. Some patients with secondary MGN may present with clinical features of MGN months or years before the manifestation of the underlying illness. Likewise, treatment with costly and potentially toxic drugs is challenging. Patients may undergo a

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series of renal biopsy to diagnose and monitor the disease condition. The discovery of anti-PLA_2R Ab [5] in the serum of primary MGN patients and anti-THSD7A Ab [6] among primary MGN variants who are seronegative for anti-PLA_2R Ab changes the dimension of diagnosis monitoring of patients using different techniques like western blot, recombinant immunofluorescence, enzyme-linked immunosorbent assay (ELISA) and many others [7]. Several studies suggested that anti-PLA_2R Ab could be used as a prognostic biomarker for primary MGN and thus, used in monitoring primary MGN patients [8,9]. In addition, most studies supported the use of ELISA as a technique of choice for the detection of biomarkers (anti-PLA_2R and anti-THSD7A) based on the following reasons: high sensitivity and specificity, ability to measure both qualitative and quantitative assays and affordability [7,10]. Despite all this progress mentioned on serum anti-PLA_2R Ab and anti-THSD7A Ab above, urine anti-PLA_2R and anti-THSD7A are lacking [7,11]. This study contributes to highlighting the role of these serum and urine biomarkers in early diagnosis, treatment decision, monitoring and prognostication of primary MGN patients.

2. Materials and Methods

2.1. Study Population and Study Design

A retrospective study (from January 2012 to October 2019) design involving biopsy-proven fully consented MGN in Hospital Serdang and Hospital Kuala Lumpur, Malaysia. Ethical approval was obtained from the National Medical Research Register (NMRR-18–3245–44092). Each subject was coded and blinded from its clinical data using a proforma to ensure confidentiality.

2.2. Methodology

2.2.1. Data Collection

Primary and secondary MGN were defined based on renal biopsy and clinical parameters. Those with associated chronic conditions like hepatitis B, hepatitis C viral infection, diabetes nephropathy, malignancy, lupus nephritis type V and many other secondary causes of MGN were deemed to be secondary MGN. In contrast, those with no known associated clinical conditions were considered as primary MGN.

A total of 125 patients were diagnosed with MGN from January 2012 to October 2019 at Hospitals Serdang and Kuala Lumpur (presented in Figure 1). General information such as age, sex and contacts of subjects were obtained from the hospitals' databases where 69 subjects agreed to participate in the study following detailed explanation, oral and written consents.

ELISA technique was used to detect the presence of biomarkers (PLA_2R, THSD7A) in serum and urine of all subjects. Urine and serum samples were measured by human anti-PLA_2R ELISA kit (Cat. No: MBS2600483) by double antibody sandwich technique while human anti-THSD7A ELISA kits (Cat. No: MBS109100) by quantitative sandwich technique. Both human PLA_2R1 and human THSD7A biomarkers were obtained from mybiosource.com. The standard curve for each of the biomarker was plotted as shown in Appendix A. In contrast, some samples were sent for laboratory tests including serum albumin, creatinine, urea, urinary protein creatinine index (UPCr Index) and estimated glomerular filtration rate (eGFR) was calculated using CKD-EPI Creatinine 2009 Equation.

2.2.2. Cut-Off Points Value for Laboratory Parameters

Complete remission and no remission were defined as <0.03 g/mmol and >0.03 g/mmol respectively according to Kidney Disease Improving Global Outcome (KDIGO) (12). Serum albumin was considered low when <35 g/L, serum urea and creatinine level were considered normal at value range 2.76–8.07 mmol/L and 44–80 μmol/L respectively. Estimated glomerular filtration rate (eGFR) was defined as follows: ≥90, 60–89, 45–59, 30–44, 15–29 and <15 mL/min/1.73 m^2 for normal, chronic kidney disease stage I (CKD 1), CKD 2, CKD 3, CKD 4 and CKD 5 respectively, calculated using CKD-EPI Creatinine

2009 Equation. The cut-off point values for PLA_2R and THSD7A were determined by taking the mean ± standard deviation of 24 normal samples and validated using receiver operating characteristic (ROC) curve: anti-PLA_2R Ab (cut-off point= 0.411 ng/mL, sensitivity = 84.6%, specificity = 100%, AOC = 1.00) and anti-THSD7A Ab (cut-off point = 0.67ng/mL, sensitivity = 90%, specificity = 100%, AUC = 1.00). The primary outcome was defined as subjects at high risk of end-stage renal disease (ESRD) (eGFR < 60) and those that could not achieve remission at the end of follow-up (UPCr Index > 0.03 g/mmol) [12].

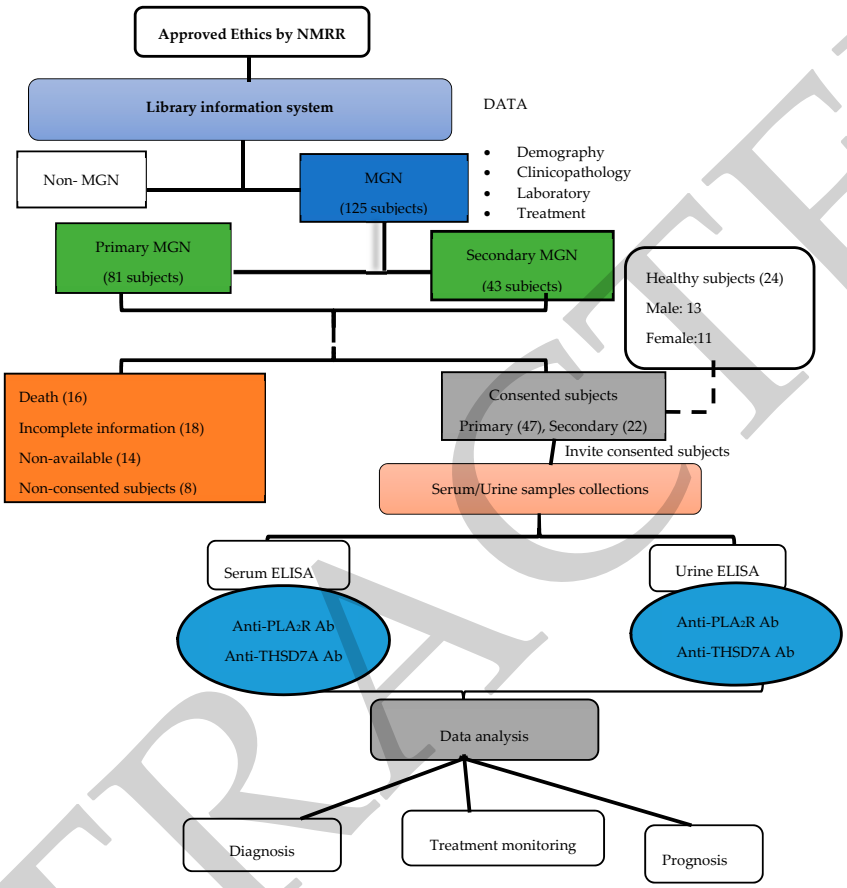

Figure 1. Flow chart of events from ethical approval to data analysis.

2.3. Data Analysis

A standard statistical software package, IBM SPSS statistics for MacBook, SPSS version 25.0 was used to analyze all the results. Normally distributed continuous variables were expressed as the mean ± standard deviation (SD) while the median (interquartile) was used for variables that were not normally distributed. Simple and multiple regression analysis was conducted to determine the prognosis, validated by the ROC curve. Differences were considered statistically significant at $p < 0.05$.

2.4. Flow Chart

3. Results

3.1. General Characteristics of the Subjects at the Time of Renal Biopsy

A total of 69 subjects, consisting of 47 primary MGN and 22 secondary MGN subjects, participated in this study. The subjects consist of 29 males and 40 females with an average age (year) of 45.5 (34.0–58.0) years, with the majority of them ≤ 30 years, most of whom were Malay and Chinese. Nephrotic syndrome was the most common presentation among primary MGN (38 of 47) subjects

while hypertension was seen among significant subjects with primary (28 of 47) and secondary MGN (10 of 22) as shown in Table 1.

Table 1. Characteristics of subjects at the time of renal biopsy.

Variable	Primary MGN (*n* = 47) Frequency	Primary MGN (*n* = 47) Percentage (%)	Secondary MGN (*n* = 22) Frequency	Secondary MGN (*n* = 22) Percentage (%)
Age at Diagnosis:				
≤30	**16**	34.1	7	31.8
31–40	**8**	17.0	4	18.2
41–50	**8**	17.0	1	4.5
51–60	**8**	17.0	4	18.2
>60	**7**	14.9	6	27.3
Sex:				
Male	21	44.7	8	36.4
Female	26	55.3	14	63.6
Ethnicity:				
Malay	24	51.1	16	72.8
Chinese	12	25.5	5	22.7
Indian	9	19.1	0	0
Others	2	4.3	1	4.5
Nephrotic Syndrome:				
Present	38	80.8	13	59.1
absent	9	19.1	9	40.9
Hypertension:				
Present	28	59.6	10	45.5
Absent	19	40.4	12	54.5
Haematuria:				
Present	9	19.1	2	9.1
Absent	38	80.9	20	90.9
Albumin:				
Normal	14	29.8	8	36.4
Low	33	70.2	14	63.6
Serum Urea(mmol/L)				
Normal	26	55.3	13	59.1
High	21	44.7	9	40.9
Serum Creatinine(μmol/L)				
Normal	6	12.8	8	36.4
High	41	87.2	14	63.6
eGFR (mL/min/1.73m^2)				
Low risk	33	70.2	15	68.2
High risk	14	29.8	7	31.8
UPCr Index(g/mmol)				
Normal	20	42.6	5	22.7
High	27	57.4	17	77.3

Estimated Glomerular Filtration Rate (eGFR) (high risk ≤ 60 mL/min/1.73m^2, low risk ≥ 60 mL/min/1.73m$^{2)}$, Urine creatinine Index (UPCr Index) (normal ≤ 0.03 g/mmol, high ≥ 0.03 g/mmol), Categorical variables were expressed as frequency and percentage.

3.2. General Characteristics of Subjects at the End of Follow-Up

Table 2 shows that after a median follow-up period of 39.0 (17.5–59.5) and 27.5 (13.0–49.8) months for both primary and secondary MGN, 14 (29.8%) and 7 (31.8%) of both primary and secondary MGN respectively were at risk of ESRD. Likewise, out of the 47 primary MGN subjects, 27 (57.4%) are yet achieve remission while only 5 (22.7%) secondary MGN subjects achieved remission.

Table 2. General characteristics of subjects at the end of the follow-up period.

Variable	Primary MGN (n = 47) Frequency	Primary MGN (n = 47) Percentage (%)	Secondary MGN (n = 22) Frequency	Secondary MGN (n = 22) Percentage (%)
Median (Interquartile)				
Follow-Up (months)	39.0(17.5–59.5)		27.5(13.0–49.8)	
eGFR(mL/min/1.73m^2)				
Low risk	33	70.2	15	68.2
High risk	14	29.8	7	31.8
UPCr Index (g/mmol)				
Remission	20	42.6	5	22.7
No remission	27	57.4	17	77.3
Biomarkers				
Serum anti-PLA$_2$R Ab (ng/mL)				
Negative	34	72.3	21	95.5
Positive	13	27.7	1	4.5
Urine anti-PLA$_2$R Ab (ng/mL)				
Negative	34	71.1	21	95.5
Positive	13	28.9	1	4.5
Serum Anti-THSD7A Ab (ng/mL)				
Negative	43	91.5	20	90.9
Positive	4	8.5	2	9.1
Urine Anti-THSD7A Ab (ng/mL)				
Negative	47	100.0	22	100.0
Positive	0	0.0	0	0.0

Categorical variables were expressed as frequency and percentage, continuous variables as interquartile.

The biomarker results from the Table 2 as follows; serum and urine anti-PLA$_2$R antibodies (Ab) were detected in 13 (27.7%) of primary MGN subjects and 1 (4.5%) for serum and urine secondary MGN (due to hepatitis B virus infection) respectively. Serum anti-THSD7A Ab was positive among 4 (8.5%) primary MGN and 2 (9.1%) secondary MGN.

3.3. Relationships Between Biomarkers and Laboratory Parameters

Table 3 described the relationship between biomarkers (PLA$_2$R and THSD7A) and respective laboratory parameters. There was a strong positive significant relationship between serum anti-PLA$_2$R Ab and urine protein creatinine index (UPCrI) (R = 0.522, $p < 0.05$) with no significant relationship between serum anti-PLA$_2$R Ab and urea, creatinine and estimated glomerular filtration rate (eGFR). Laboratory parameters such as urea (R = 0.251, $p < 0.038$) and UPCr Index (R = 0.437, $p < 0.05$)

were, respectively, poorly and fairly correlated with urine anti-PLA_2R Ab. Urine anti-PLA_2R Ab and serum anti-PLA_2R Ab were highly correlated ($R = 0.902$, $p < 0.05$). There was no correlation between serum anti-THSD7A Ab and urine anti-THSD7A Ab and other laboratory parameters. The graphical presentations of some of the variables with significant correlation are presented in Appendix B.

Table 3. Relationship between biomarkers and laboratory biomarkers.

Covariate	R	*p*-Value
Serum anti-PLA_2R Ab (ng/mL)		
Urine anti-PLA_2R Ab (ng/mL)	0.902	<0.05 *
Albumin (g/L)	0.056	0.647
Urea (mmol/L)	0.226	0.062
Creatinine (µmol/L)	0.034	0.782
eGFR (mL/min/1.73m^2)	0.216	0.075
UPCr Index (g/mmol)	0.502	<0.05 *
Urine anti-PLA_2R Ab (ng/mL)		
Albumin (g/L)	0.040	0.746
Urea (mmol/L)	0.251	0.038 *
Creatinine (µmol/L)	0.550	0.652
eGFR (mL/min/1.73m^2)	−0.08	0.514
UPCr Index (g/mmol)	0.437	<0.05 *
Serum anti-THSD7A Ab (ng/mL)		
Urine anti-THSD7A Ab (ng/mL)	0.063	0.604
Albumin (g/L)	−0.069	0.574
Urea (mmol/L)	−0.137	0.999
Creatinine (µmol/L)	−0.110	0.367
eGFR (mL/min/1.73m^2)	0.136	0.266
UPCr Index (g/mmol)	−0.069	0.395

eGFR = estimated glomerular filtration rate, UPCr index = urine protein creatinine index. Correlation (*R*) given as <0.25 as poor, 0.26–0.5 as fair, 0.51–0.75 as good, and >0.75 as excellent. Level of significance <0.05 *.

3.4. The Prognostic Outcome of Primary MGN Subjects

3.4.1. The Prognostic Outcome of Primary MGN using eGFR (CKD ≥ 3)

Most of the variables except for sex, albumin and serum anti-THSD7A were retained following simple logistic regression described in Table 4 below. However, variables like urine anti-PLA_2R Ab and serum creatinine were excluded due to multicollinearity. Following multiple logistic regression, only urea (B = −1.174, S.E. = 0.441, 95% C.I. = 0.130–0.734, AOR = 0.309, $p = 0.008$) and serum anti-PLA_2R Ab (B = −1.447, S.E. = 1.467, C. I. = 0.013–4.175, AOR = 0.235) as shown in Table 5. Urea increases the risk of the primary outcome (CKD ≥ 3) by the odd of 0.309 (69.1%) for any unit increase in urea (95 % C.I.) while high serum anti-PLA_2R Ab titre increased the risk of the primary outcome (CKD ≥ 3) by 4.3 odds compared with those subjects with normal serum anti-PLA_2R Ab titre. The result was validated by receiver operating characteristics (ROC) curve (AUC = 0.960, sensitivity = 87.0%, specificity = 93.3%) as shown in Figure 2.

3.4.2. The Prognostic Outcome of Primary MGN using UPCr Index (Remission)

Factors associated with remission were assessed as important prognostic factors. Simple logistic regression was used where creatinine (SE = 0.015, COR = 1.047, 95% CI = 1.016–1.079, $p = 0.002$), eGFR(SE = 0.013, COR = 0.954, CI = 0.931–0.978, $p < 0.05$) and urine anti-PLA_2R Ab(SE = 1.249, COR = 11.845, 95% CI = 1.025–136.924, $p = 0.048$) were found to be significant as shown in Table 6, while Table 7 shows multiple regression validated by ROC curve in Figure 3 (AUC=0.890, sensitivity= 82.9%, specificity= 94.1%); only urine anti-PLA_2R Ab was retained as a primary factor associated

with remission. Therefore, any rise in one unit of urine anti-PLA_2R Ab titre increases the risk of not achieving remission by 18.486% (95% C.I. = 0.153–2234.834, $p < 0.05$).

Table 4. Simple logistic regression for the prognostic outcome of primary MGN using eGFR (CKD ≥ 3).

Variables	B	SE	COR	CI (95%)	*p*-Value
Age	0.062	0.023	0.940	0.898-0.993	0.008 *
Gender					
Male			1.000		
female	0.241	0.587	1.273	0.403–4.021	0.681
Albumin (g/L)	0.111	0.077	1.117	0.961–1.299	0.149
Urea (mmol/L)	0.883	0.259	2.417	1.454–4.019	0.002 *
Creatinine (μmol/L)	0.178	0.068	1.195	1.046–1.316	0.009 *
UPCr Index (g/mmol)					
Remission			1.000		
No remission	−2.565	0.813	0.077	0.016–0.378	0.002 *
Serum anti-PLA_2R Ab(ng/mL)					
Negative			1.000		
Positive	−1.978	0.971	0.138	0.021–0.928	0.042 *
Urine anti-PLA_2R Ab(ng/mL)					
Negative			1.000		
Positive	−1.833	0.755	0.160	0.036–0.703	0.015 *
Serum anti-THSD7A Ab(ng/mL)					
Negative			1.000		
Positive	1.872	0.967	6.500	0.976–43.289	0.053

SE = Standard error, COR = Crude Odd Ratio, CI = Confidence Interval, level of significant $p < 0.05$ *.

Table 5. Multiple logistic regression for the prognostic outcome of primary MGN using eGFR (CKD ≥ 3).

Variables	B	SE	AOR	C.I. 95%	*p*-Value
Age at diagnosis (years)	−0.094	0.052	0.910	0.822–1.009	0.534
Serum anti-PLA_2R Ab(ng/mL)	−1.447	1.467	1.000 0.235	0.013–4.175	0.048 *
Urea(mmol/L)	−1.174	0.441	1.000 0.309	0.130–0.734	0.008 *

SE = Standard Error, AOR= Adjusted Odd Ratio, C.I. = Confidence Interval, level of significance, $p < 0.05$ *.

Table 6. Simple logistic regression for the prognostic outcome of primary MGN using UPCr Index (Remission).

Variable	B	SE	COR	95% C.I.	*p*-Value
Age at diagnosis	0.025	0.018	1.025	0.989–1.063	0.181
Sex	0.308	0.490	1.360	0.521–3.551	0.530
Albumin(g/L)	−0.075	0.060	0.928	0.825–1.043	0.209
Urea(mmol/L)	0.257	0.136	1.293	0.991–1.686	0.058
Creatinine (μmol/L)	0.046	0.015	1.047	1.016–1.079	0.002 *
eGFR(mL/min/1.73m^2)	−0.047	0.013	0.954	0.931–0.978	<0.05 *
Serum anti-PLA_2R Ab (ng/mL)	1.377	0.810	3.964	0.810–19.399	0.015 *
Urine anti-PLA_2R Ab(ng/mL)	2.472	1.249	11.845	1.025–136.924	0.05 *
Serum anti-THSD7A Ab(ng/mL)	−0.937	1.022	0.392	0.530–2.902	0.392

SE = Standard Error, COR = Crude Odd Ratio, C.I. = Confidence Interval, level of significance <0.05 *.

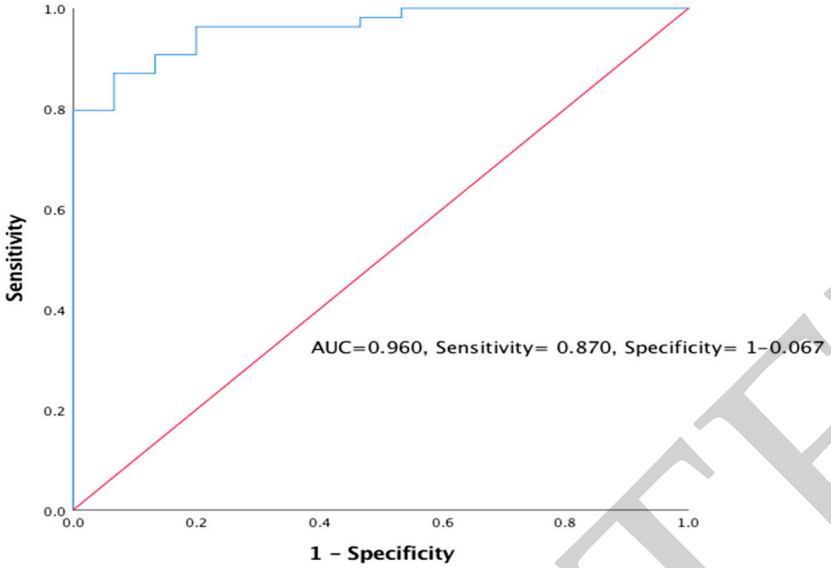

Figure 2. Receiver operating characteristics (ROC) curve was used to validate predictors of primary outcome using eGFR (CKD ≥ 3).

Table 7. Multivariate logistic regression for the prognostic outcome of primary MGN using UPCr Index (Remission).

Variable	B	SE	AOR	95% C.I.	*p*-Value
Creatinine	0.018	0.044	1.018	0.934–1.110	0.660
eGFR (mL/min/1.73m^2)	0.002	0.039	1.002	0.928–1.882	0.958
Urine anti-PLA$_2$R Ab (ng/mL)	2.917	2.446	18.486	0.153–2234.834	0.038

SE = Standard Error, AOR = adjusted odd ratio, C.I. = Confidence Interval, level of significance <0.05 *.

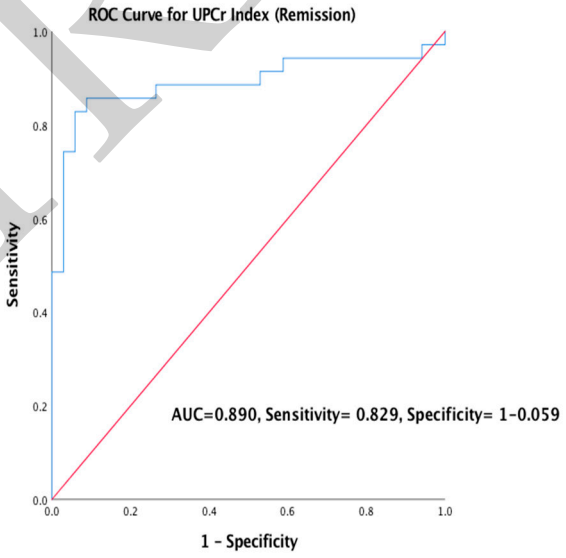

Figure 3. ROC Curve was used to validate the predictors of outcome using UPCr Index (Remission).

4. Discussion

Discovery of serum biomarkers for MGN has been part of exceptional achievement in the management of primary MGN patients. These biomarkers can help in making the diagnosis, making a decision based on whether to give supportive or immunosuppressive therapy or both. The importance

of serum and urine biomarkers (anti-PLA_2R Ab and anti-THSD7A Ab) in the management of primary MGN patient was emphasized in this study.

A total of 69 subjects consisting of 47 primary and 22 secondary MGN fully consented and enrolled in this study with an average follow-up of 36.0 (15.0–57.0) in months.

4.1. Role of Biomarkers (PLA_2R and THSD7A) in the Diagnosis of MGN

A high index of suspicion is needed from history and clinical presentation to rule out secondary causes before making a diagnosis of primary MGN and confirm by renal biopsy. However, this might be time-consuming, and patients on an anticoagulant may have delayed biopsy. For these reasons, biomarkers like serum and urine anti-PLA_2R Ab complimented by serum anti-THSD7A Ab were employed to make the diagnosis of MGN without waiting long for the manifestation of other features [6,13]. From this study, 13 of the 47 biopsy-proven primary MGN patients were found to be positive for both serum and urine anti-PLA_2R Ab at the end of follow-up. In contrast, a serum and urine sample of the same subject associated with hepatitis B virus was positive for secondary MGN at the end of follow-up. Although anti-PLA_2R Ab was considered a reliable biomarker for primary MGN [5], its appearance in secondary MGN was not surprising since the biomarker was reported to be associated with hepatitis B virus infection [14,15]. A biomarker, anti-THSD7A Ab can be used to make a diagnosis of primary MGN variants not detected by anti-PLA_2R Ab [16]. In this study, serum anti-THSD7A Ab was positive in four biopsy-proven primary MGN subjects and two secondary MGN subjects (one of which was associated with malignancy and the other was due to lupus nephritis type V). This result may be possible for the fact that anti-THSD7A Ab was associated with malignancy [17–19] and we can't also rule out the presence of malignancy or lupus nephritis coexisting with primary MGN. Therefore, malignancy should be suspected among anti-THSD7A Ab positive subjects.

4.2. Role of Biomarkers in Monitoring MGN Subjects

It was widely reported that biomarker like PLA_2R Ab could be used to monitor subjects with primary MGN [20,21]. Therefore, positive detection of anti-PLA_2R Ab in 13 subjects and anti-THSD7A Ab in four subjects with biopsy-proven primary MGN after a prolonged period of follow-up justified the importance of these biomarkers in monitoring MGN. Studies have shown that changes in biomarker titre are immunological, and the depletion of anti-PLA_2R Ab and an increase in serum albumin level preceded the decrease in proteinuria. Therefore, anti-PLA_2R Ab can predict remission better than proteinuria [9,22]. The above finding is another reason why anti-PLA_2R Ab is needed to monitor patients with primary MGN.

4.3. Prognosis of MGN

From Table 3 above, it was demonstrated that there was a significant positive relationship between anti-PLA_2R Ab and UPCr Index, thereby confirming the fact that those biomarkers can be used to determine prognosis since they are significantly associated with prognostic laboratory parameters [23].

Anti-PLA_2R Ab as a Prognostic Biomarker for Primary MGN

An elevated anti-PLA_2R Ab titre is associated with poor prognosis and increased risk of progression to ESRD [9] or post kidney transplantation failure [24–26] whereas low anti-PLA_2R Ab titre is associated with spontaneous remission [21,27–29]. Therefore, the low prevalence of anti-PLA_2R Ab at the end of follow-up in this study demonstrated that anti-PLA_2R Ab is a prognostic biomarker for primary MGN as against UPCr Index level, which showed fewer subjects achieving remission at the end of follow-up. Thus, anti-PLA_2R Ab could help in deciding when to commence or stop immunosuppressive therapy and also those patients that may likely have ESRD.

5. Conclusions

Serum (anti-PLA_2R Ab and anti-THSD7A Ab) and urine anti-PLA_2R Ab are essential biomarkers for diagnosis, treatment decision and prognosis of patients with primary MGN. In addition, biomarkers should be clinically recommended to make a diagnosis of primary MGN in patients who were unable to undergo renal biopsy and to monitor the response to treatment.

Author Contributions: The conceptualization of this study was done by S.M.M. and F.A.G.; methodology, S.M.M. and F.A.G.; validation, N.F.Z., F.A.G. and R.H.; formal analysis, S.M.M.; investigation, F.Z. and S.M.M.; resources, F.A.G.; data curation, S.M.M.; writing—original draft preparation, S.M.M.; writing—review and editing, F.Z., R.H. and F.A.G.; visualization, S.M.M.; supervision, F.A.G., R.H. and F.Z.; project administration, F.A.G.; funding acquisition, F.A.G. and F.Z. All authors have read and agreed to the published version of the manuscript.

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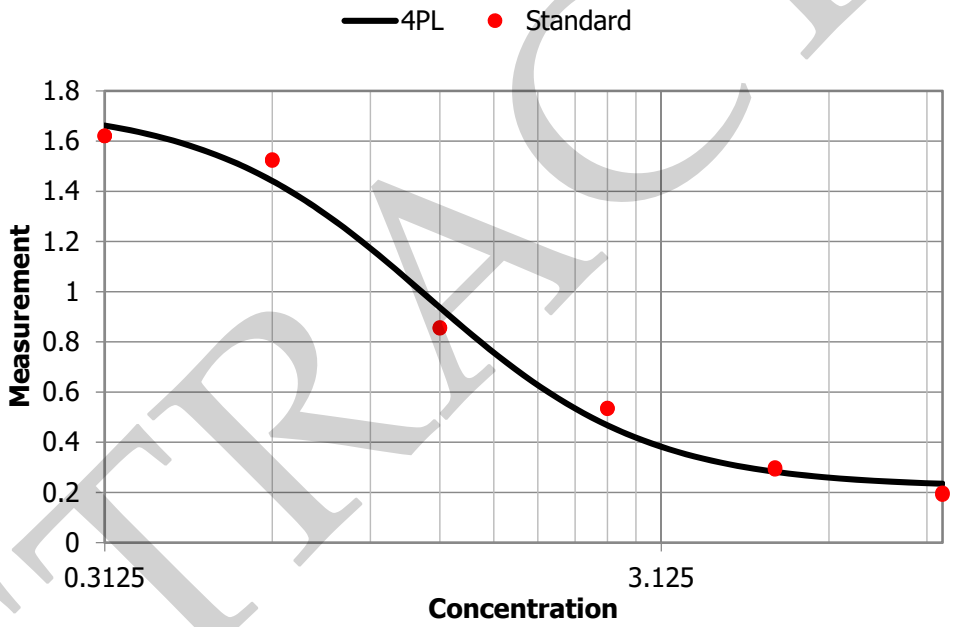
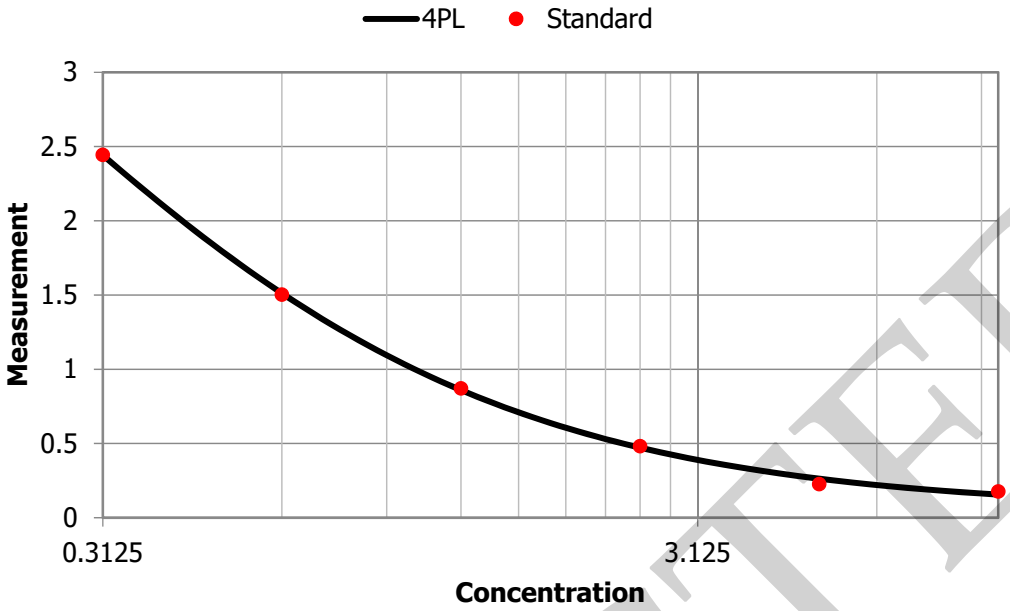
Appendix A



a	0.2209
b	−2.203
c	1.188
d	1.738
MSE	0.003579
R^2	0.9886
SS	0.04294
SYX	0.07327

Figure A1. Anti-PLA_2R ELISA standard curve.



a	0.05272
b	−1.051
c	0.2162
d	5.961
MSE	0.0003364
R^2	0.9995
SS	0.002018
SYX	0.03177

Figure A2. Anti-THSD7A standard curve.

Appendix B

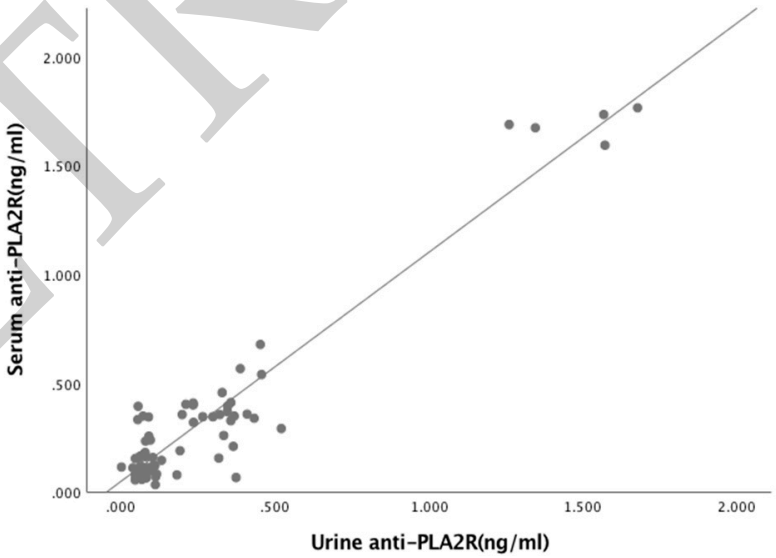


Figure A3. Relationship between Serum anti-PLA_2R and urine anti-PLA_2R (R = 902, $p < 0.001$).

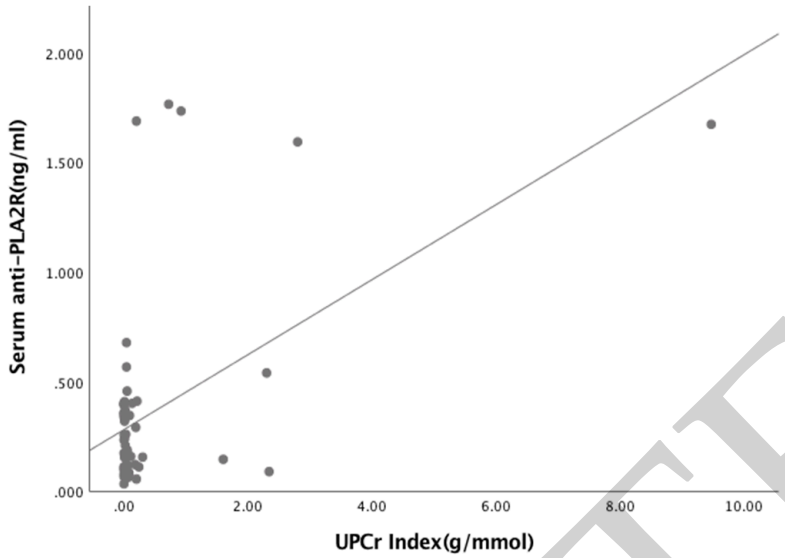


Figure A4. Relationship between Serum anti-PLA_2R and UPCr Index (R = 0.502, $p < 0.001$).

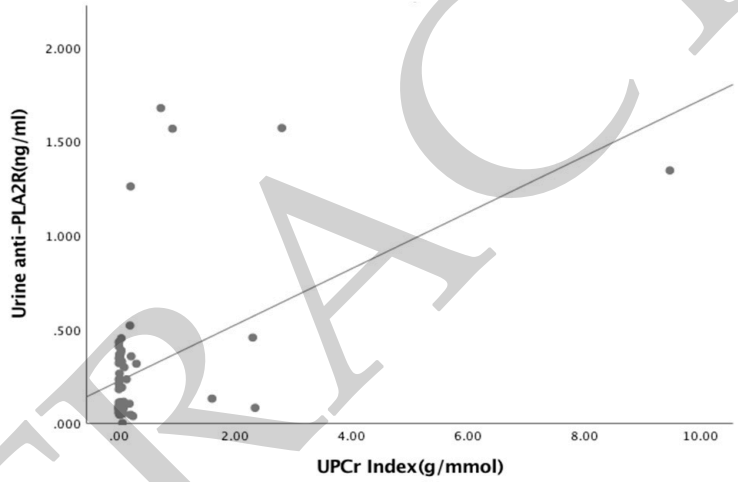


Figure A5. Relationship between Urine anti-PLA_2R and UPCr Index (R = 437, $p < 0.001$).

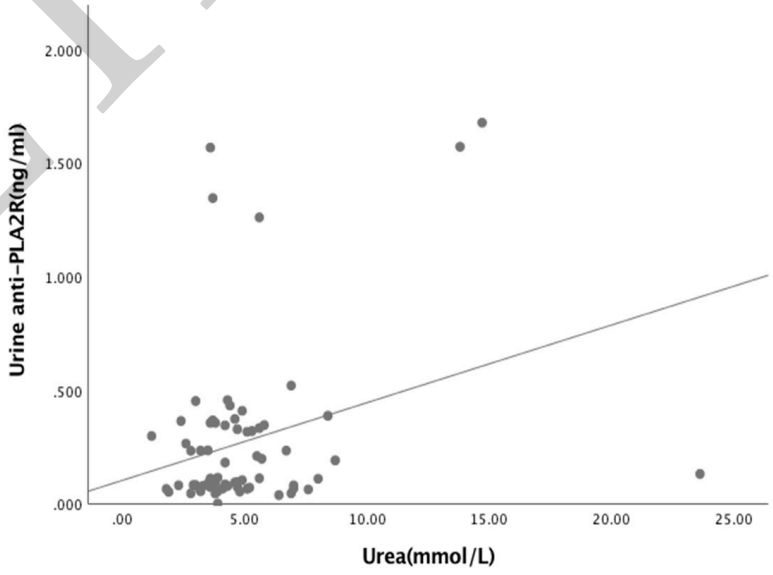


Figure A6. Relationship between Urine anti-PLA_2R and serum Urea (R = 0.201, $p < 0.038$).

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