Università degli Studi di Messina

DEPARTMENT OF BIOMEDICAL, DENTAL, MORPHOLOGICAL AND FUNCTIONAL IMAGING SCIENCES

Translational Molecular Medicine and Surgery - XXXVII PhD Cycle

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A MULTICENTER OBSERVATIONAL STUDY ON PREDICTIVE FACTORS OF IMMUNOSUPPRESSIVE THERAPY EFFICACY IN MEMBRANOUS NEPHROPATHY

(SSD MED-08/B)

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Academic Year 2023/2024

Summary

1. Introduction

Personalized medicine represents a paradigm shift in treating patients more than diseases, to go beyond the hypothesis that "one size fits all". Glomerular diseases are included in this consideration for choosing the most suitable therapeutic agent and its appropriate dose, because a standardized treatment may result in worse patient outcomes, including rapid deterioration of kidney function up to end-stage renal disease (ESRD) or life-threatening acute complications.

The aim of the PhD project conducted during the XXXVII° Cycle of Translational Molecular Medicine and Surgery was to identify clinical and immunologic features which may influence the response to the treatment of glomerular diseases.

In particular, we directed our attention to the potential association of obesity at diagnosis with remission rate in patients affected by membranous nephropathy (MN), which represents one of the leading causes of primary nephrotic syndrome in adults.

Obesity is a known risk factor for chronic kidney disease (CKD), since it has been shown to accelerate the progression of renal damage[1] and rejection after kidney transplantation. In addition, the incidence of glomerulopathies has increased in conjunction with the global obesity epidemic. This association appears to be related to elevated intraglomerular pressure leading to hyperfiltration and to augmented metabolic demand in obese patients' nephrons[2]. The equivalent

anatomic finding is glomerulomegaly, recognisable in kidney biopsy in obesity-related glomerulopathy (ORG) and may lead to a "maladaptive" secondary form of focal segmentary glomerulosclerosis (FSGS) in the same way in patients with reduced renal mass[3].

The prospective study NEPTUNE[4], conducted on 541 subjects suffering from primary proteinuric glomerulonephritis, has investigated the association between obesity, glomerulopathies, chronic renal damage and cardiovascular risk, trying to define some outcome markers. In adults, obesity, assessed through the body mass index (BMI) was associated with hypertension and dyslipidemia and with a lower probability of achieving complete remission despite the treatment of the underlying disease. The NEPTUNE study also hypothesized that obesity acts directly on glomeruli, modifying the architectural structure and thus influencing the clinical history of the disease and ability to achieve remission. Secondly, obese patients experience a different metabolism of some immunosuppressive drugs, with an impact on their therapeutic benefit[5,6].

In opposition to other published studies, we aimed to longitudinally analyze the role of body mass index (BMI) and the impact of the relationship between proteinuria and BMI in modifying renal outcomes of nephrotic syndrome due to membranous nephropathy treated with immunosuppressive drugs.

Our study started with the Glomerular Disease outpatient clinic in the Nephrology and Dialysis Unit of University Hospital G. Martino in Messina, where MN was among the most frequent diagnoses after kidney biopsy in suspected glomerulonephritis.

The first nucleus of the study, was accepted and presented as an abstract at European Renal Association/European Dialysis and Transplant Association (ERA/EDTA) congress in 2020[7]; an expanded cohort was accepted as a Mini Oral presentation at European Renal Association/European Dialysis and Transplant Association (ERA/EDTA) congress in 2022[8].

The retrospective revision of clinical charts was extended later to the Nephrology and Dialysis of the Spedali Civili in Brescia, enlarging our sample size to reach statistical significance and confirm our hypothesis.

2. Membranous Nephropathy: a problem of clinical relevance

MN represents the most common cause of nephrotic syndrome worldwide after diabetes. The global incidence of 8-10 cases/million/year has a peak in males with age comprised between 40 and 60 years old[9].

Primary forms (75-80% of MN cases) are characterized by glomerular sub-epithelial deposition of IgG immunocomplexes, mainly directed against M-type phospholipase-A2-receptor (PLA2R) expressed on the podocytes' surface, and require immunosuppressive therapy. Secondary forms account for 20-25% of patients and are usually due to systemic autoimmune disorders, infections, diseases, malignancies or triggered by drugs. In these cases, targeting the primitive disorder resolves nephrotic syndrome[10,11].

Only 30% of patients have a mild clinical presentation with sub-nephrotic proteinuria. About 70% of MN patients develop nephrotic syndrome, with a degree of proteinuria greater than 3.5 g/day, hypoalbuminemia, and fluid overload ranging from mild oedemas up to anasarcatic state (including ascites, pleural and pericardial effusion).

Patients with nephrotic syndrome are predisposed to significant dyslipidemia with increased total and low-density (LDL)-cholesterol and reduced high-density (HDL)-cholesterol, conferring high atherogenic risk.

Moreover, the liver compensates for anticoagulant and profibrinolytic factors' loss through the glomerular filtration barrier with increased synthesis of procoagulant proteins such as fibrinogen, factor V, factor VIII, determining hypercoagulability and deep venous as well as arterial thromboembolism.

The disease course is variable, with patients' risk assessment established by 2021 Kidney Disease Improving Global Outcomes (KDIGO) Glomerular Diseases guidelines (table 1)[12]. Up to 30% of patients experience mild symptoms at the onset of MN with normal renal function, sub-nephrotic proteinuria, and respond to first-line therapy with antiproteinuric agents such as angiotensin-converting enzyme inhibitors (ACEi) or angiotensin II receptor blocker (ARB). This group is characterized by a low risk of CKD and may remit within two years since diagnosis. By contrast, sustained nephrotic range proteinuria may lead to a chronic kidney injury and even to end-stage renal disease (ESRD) requiring renal replacement therapy[10-12].

Patients at high risk of progression to renal failure over time are identified by reduced glomerular filtration rate (GFR) at diagnosis, missed partial remission after six months of conservative treatment with ACEi or ARB associated with persistent hypoalbuminemia, high title anti PLA2R antibodies (> 50 RU/ml), or increased IgG, α1 or β2-microglobulin urinary excretion[12].

The abrupt onset of nephrotic syndrome and a rapid deterioration of eGFR are strongly predictive of reduced renal survival, due to the irreversible loss of functioning nephrons, and require immediate treatment with immunosuppressive agents to avoid life-threatening complications[13,14].

The treatment options in high and very high-risk MN patients include rituximab, a chimeric mouse/human monoclonal antibody targeting B cells' surface antigen CD20, or cyclophosphamide and alternate month glucocorticoids for six months (Ponticelli regimen). In case of moderate risk of progression, the clinicians may evaluate rituximab or a 6 to 12-month course of calcineurin inhibitors (CNI) in association with glucocorticoids, given the elevated incidence of relapse after CNI monotherapy[12].

Low risk	Moderate risk	High risk	Very high risk
1. Normal eGFR 2. Subnephrotic proteinuria (< 3.5 g/day) or at least 50% reduction after 6 months of antiproteinuric therapy (ACEi or ARB) 3. Serum albumin > 30 g/l	1. Normal eGFR 2. Nephrotic proteinuria	1. eGFR<60 ml/min/1.73m^2 or proteinuria > 8 g/day for at least 6 months **OR** 2. Normal eGFR, nephrotic proteinuria and < 50% reduction after 6 months of antiproteinuric therapy AND o Serum albumin < 25 g/dl o PLA2Rab > 50 RU/ml o Urinary IgG > 1 µg/min o Urinary α1-microglobulin > 40µg/min o Urinary β2- microglobulin > 250 mg/day o Selectivity index (IgG/albumin clearance) > 0.20	1. Life-threatening nephrotic syndrome 2. Rapid deterioration of eGFR not otherwise explained

Table 1 Clinical criteria for assessing risk of CKD progression in patients affected by MN (adapted from 2021 KDIGO Glomerular Diseases guidelines)[12].

ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker; eGFR, estimated glomerular filtration rate; IgG, immunoglobulin G; PLA2Rab, anti-M-type phospholipase A2 receptor antibodies.

2.1 Primary membranous nephropathy

Debiec et al described neutral endopeptidase (NEP) as the first human antigen targeted in a case of antenatal MN. The mother resulted deficient of this enzyme and immunization occurred during pregnancy, with the transplacental passage of antibodies and their deposition on the fetal glomerular basement membrane[15].

The discovery of podocyte M-type phospholipase-A2-receptor (PLA2R) as the most common antigen responsible for the disease (about 75% of cases), represented a turning point for MN diagnosis in 2009. In particular, autoantibodies were soon detectable thanks to an enzyme-linked immunosorbent assay (ELISA) or indirect immunofluorescence assay (IFA) in patients'serum and in kidney biopsy samples also giving the chance of monitoring "immunologic" response to therapy or the relapse even before developing clinical evidence[16].

PLA2R antibodies should be measured every three or six months, preferring the shorter interval in patients with high titers at baseline. Changes in PLA2R antibodies levels during follow-up may modify risk estimation during the course of the disease. Moreover, the disappearance of anti-PLA2R antibodies usually precedes clinical remission and may lead to refraining from additional therapy[12].

Thrombospondin type 1 domain-containing 7 A (THSD7A) was later discovered as the antigen involved in 5% of MN, but in the remaining 20% of seronegative primary forms the antigens are yet to be completely identified[17].

New evidence is coming after the identification of circulating and glomerular autoantibodies directed against neural EGF-like-1 protein (NELL-1) in primary

MN. NELL-1 is expressed in 5%–25% of glomerular cells mRNA, but remarkably, a subgroup of 10-33% of positive patients may however develop a malignancy[18,19].

Semaphorin 3B (Sema3B) has been recognized in 73% of the rare paediatric forms of primary MN and in fewer cases in young adults, globally accounting for 1-3% of all cases of MN but which often have a familial recurrence, raising the hypothesis of a genetic predisposition. Moreover, the circulating autoantibodies against Sema3B may represent a practical aid for the correct differential diagnosis of primary nephrotic syndrome in children, because MN could be initially treated as a steroid-resistant nephrotic syndrome if a kidney biopsy is not performed[20-22].

Protocadherin 7 (PCDH7) is a signalling protein belonging to the cadherin family, recently identified as a possible MN antigen in the elderly. Autoantibodies against PCDH7 can also be detected in serum and the related MN form is apparently characterized by a favourable prognosis without the need for immunosuppressive treatment[22,23].

The last antigen discovered is High-Temperature Recombinant Protein A1 (HTRA1), a ubiquitary serin protease which may be the target in older patients. It currently accounts for about 1-2% of primary MN[24].

2.2 Autoimmunity in secondary membranous nephropathy

As regards secondary MN, particular attention has to be paid to the identification of autoantibodies against glycosyltransferases exostosin 1/exostosin 2 (EXT1/EXT2) in kidney biopsies of young adults. In particular, EXT1/EXT2 positive MN may accompany or anticipate the systemic manifestation of connective tissue diseases or systemic lupus erythematosus (SLE)[25].

Neural cell adhesion molecule 1 (NCAM1) is a glycoprotein mainly expressed in the nervous, hematopoietic system and, to a lesser degree, in podocytes and renal interstitial cells. Recent observations demonstrated the deposition of autoantibodies directed against NCAM1 on the glomerular basement membrane in class V lupus nephritis, that shares indeed common pathology features with MN[26].

Transforming Growth Factor Beta Receptor 3 (TGFBR3) is a transmembrane proteoglycan diffused in glomeruli as it is expressed in podocytes, mesangial and endothelial cells. Interestingly, it shares features with NCAM1 and EXT1/2 patients, with the majority of them diagnosed with other autoimmune diseases. It affects mainly young women with mixed membranous and proliferative marks[27].

Several case reports have described the concurrence of chronic inflammatory demyelinating polyneuropathy (CIDP) and MN, but the co-existence of autoantibodies against paranodal proteins such as contactin-1 (CNTN1) in subepithelial deposits has been demonstrated by Santoro et al and may be a novel target of immunosuppressive treatment[28].

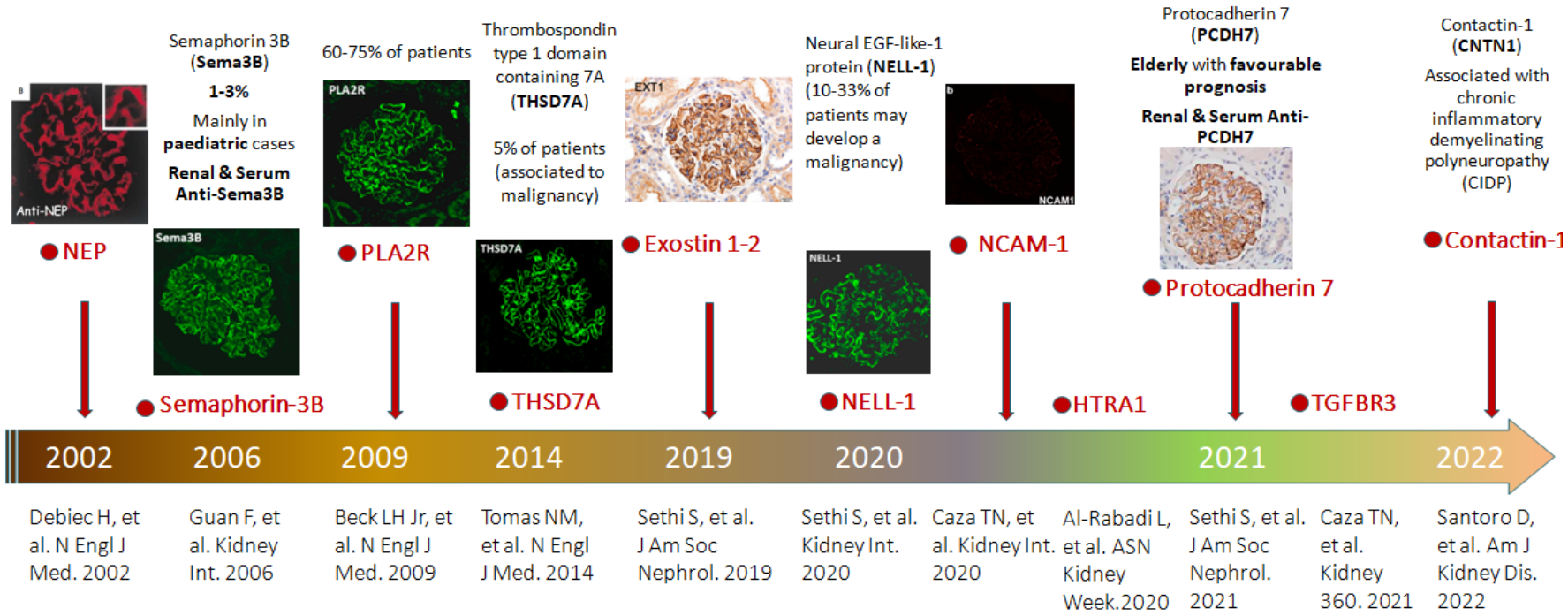

Figure 1 Timeline of antigen discovery in primary and secondary MN. Courtesy of Prof. Domenico Santoro (2021) and updated.

HTRA, High-Temperature Recombinant Protein A1; NCAM-1, Neural cell adhesion molecule 1; NELL-1, Neural EGF-like 1protein; NEP, neutral endopeptidase; PLA2R, M-type phospholipase-A2-receptor; TGFBR3, Transforming Growth Factor Beta Receptor 3.

3. Methods

3.1 Population and laboratory data

We recruited patients with biopsy-proven diagnosis of membranous nephropathy in a period from January 2005 to December 2020, afferent to the ward of Nephrology and Dialysis of the University Hospital G. Martino in Messina (Italy) and the ward of Nephrology and Dialysis of the Spedali Civili in Brescia (Italy).

Patients included in our study underwent quarterly controls for at least 12 months and the last follow-up examination happened between 2016 and 2020. The principal clinical features considered were weight, height, systolic and diastolic pressure. We included into analysis complete blood count, serum creatinine, urea, triglycerides, total cholesterol and HDL-c, urine exam, 24-hour proteinuria, and determined estimated GFR (eGFR using the CKD-EPI formula)[29]. We also reviewed the clinical history of patients recording domiciliary therapy and comorbidities.

3.2 Statistical analysis

The distribution of variables was assessed using the Kolmogorov-Smirnov test and graphic evaluation. Baseline features were analyzed using the T-student test for independent variables, the Mann-Whitney test for continue variables according to distribution, and the Pearson's Chi-Square analysis for dummy variables.

Correlations were detected using the Pearson or Spearman's correlation tests using proteinuria/BMI ratio as continue variables.

Trend analyses were computed with a Linear Mixed Model (LMM) for repeated analysis, using as the random variable the number of visits (time) and ID number to discriminate all patients. In the unadjusted model, the interaction between proteinuria/BMI ratio and time (Time*proteinuria/BMI) was examined. Confounding variables in the adjusted model were chosen following clinical criteria (age, sex, hypertension, diabetes mellitus, treatment) or if statistical association with outcome variables or treatment had $\alpha \leq 10\%$ ($P \leq 0.1$). The linear combination was performed to detect the impact of the effect modifier. Residual studies were used to accept or reject models. Statistical analysis was performed using SPSS version 24 (version 24.0; IBM Corporation, Armonk, NY, USA) and STATA version 13 (Stata Corporation, College Station, TX).

4. Results

Our study included 435 repeated measurements in 87 total patients. The mean age of our population was 54±18 years old, with 28 males and 59 females. Median eGFR and proteinuria were, respectively, 75 ml/min/1.73m^2 [IQR 51-104 ml/min/1.73m^2] and 4.9 g/day [IQR 2.4-8.0 g/day].

The status of PLA2R-ab at diagnosis was marked according to the indications of the ELISA laboratory kit for analysis, which considered the cut-off for negative values < 14 RU/ml (22 patients), borderline values ranging from 14 to 20 RU/ml (4 patients), positive values > 20 RU/ml (34 patients). PLA2R-ab titer was not available at diagnosis in the remaining 27 patients.

The revision of clinical charts detected baseline proteinuria/BMI ratio in only 61 patients at diagnosis, who were included in our statistical analysis. The distribution of baseline eGFR is shown in Figure 2.

The whole cohort was split into two subgroups according to their basal proteinuria/BMI ratio being lower or higher than the median value. Arterial hypertension (30% vs 16%, *P*=0.23), higher levels of LDL-c (174±64 mg/dl vs 159±61 mg/dl, *P*=0.55) and triglycerides (190±123 mg/dl vs 181±85 mg/dl, *P*=0.76) were prevalent in the subgroup with lower proteinuria/BMI ratio. However, this pattern represented a trend without reaching a statistical significance.

Recurrent comorbidities, apart from hypertension, were thyroiditis, arthritis, atherosclerosis, diabetes, sarcoidosis and celiac disease. No significant differences were highlighted according to the proteinuria/BMI ratio. Table 2 resumes baseline features in the subgroups.

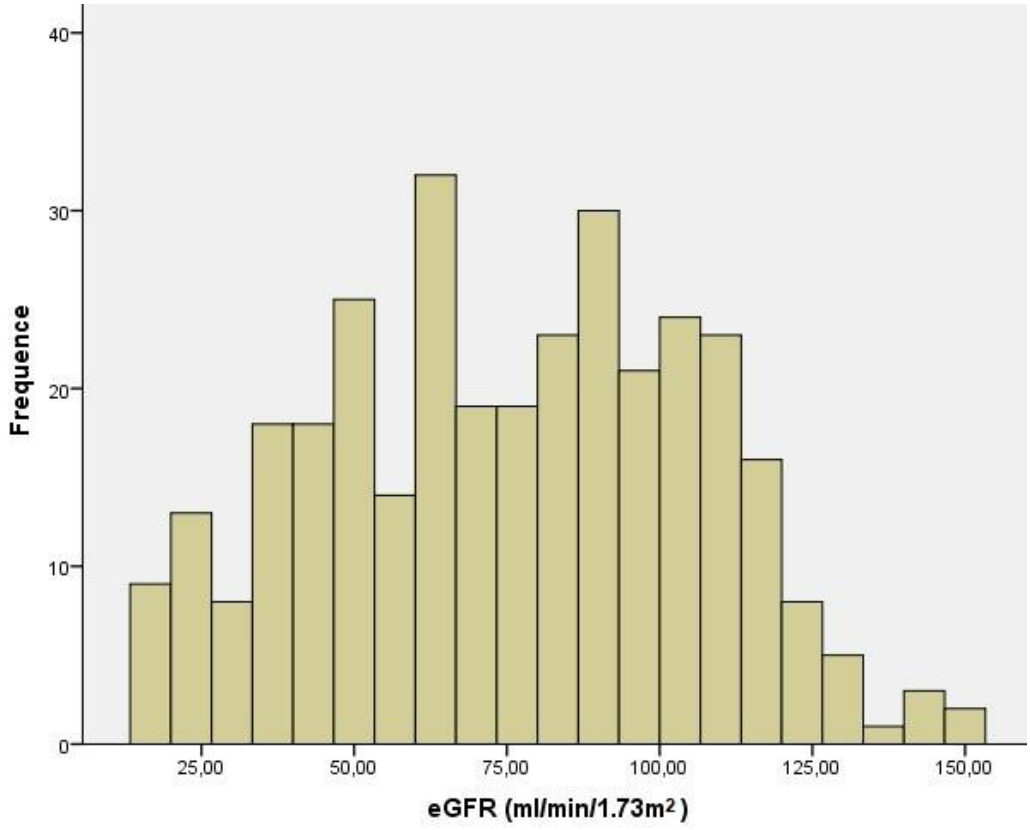

Figure 2. Distribution of eGFR (ml/min/1.73 m^2) values at biopsy time in the sample of patients with membranous nephropathy

Variable	24h proteinuria/BMI ratio		*P*	Correlation coefficient
	Lower than median N= 30	Higher than median N=31		
Age, years	56±15	49±21	0.15	0.15
Sex, Male (%)	**13 (43%)**	**6 (19%)**	**0.06**	**-0.23***
Sclerotic glomeruli, n.[IQR]	**1 [1-3]**	**1[0-2]**	**0.04**	**-0.33***
Systolic blood Pressure, mmHg [IQR]	133 [120-144]	130 [119-140]	0.34	0.22
Diastolic blood pressure, mmHg [IQR]	76 [70-81]	80[70-80]	0.98	-0.01
Haemoglobin, g/dl	12.8±1.8	13.0±1.5	0.76	-0.09
PTL, cc*10^3/ml [IQR]	220 [103-288]	266[197-316]	0.29	0.16
Albumin, g/dl*	**3.0±0.7**	**2.2±0.6**	**0.001**	**-0.68***
Urinary RBC cc/mmc	0 [0-4]	3 [0-7]	0.11	0.16
LDL, mg/dl	174±64	159±61	0.55	0.04
Triglycerides, mg/dl	190±123	181±85	0.76	0.09
eGFR, ml/min/1.73 m^2	82±30	87±29	0.52	0.15
Arterial hypertension, n. (%)	9 (30%)	5 (16%)	0.23	0.04
RAASi, n. (%)	15 (58%)	17 (57%)	0.94	-0.10
Thyroiditis, n. (%)	4 (13%)	4 (13%)	0.76	0.05
Arthritis, n. (%)	13 (43%)	15 (48%)	0.69	0.06
Atherosclerosis, n. (%)	4 (13%)	2 (6.5%)	0.43	0.13
Diabetes mellitus, n. (%)	3 (10%)	4 (13%)	1.00	0.09
Sarcoidosis, n. (%)	3 (10%)	1 (3.2%)	0.35	0.10
Vascular encephalopathy, n. (%)	0 (0%)	1 (3.2%)	1.00	0.05
Celiac disease, n. (%)	4 (13%)	0 (0%)	0.06	0.14

Table 2. Summary of features at first visit. Data are summarized as Mean ± standard deviation, Median [Interquartile range] or N° (%) as appropriate. eGFR, estimated glomerular filtration rate; LDL, Low density lipoprotein; PLA2R-Ab, phospholipase A 2 receptor antibodies; PTL: Platelets; RAASi, Renin-angiotensin-aldosterone-system inhibitors.

Significant associations were found between the proteinuria/BMI ratio and sex (Rho= -0.23, *P*=0.06), baseline serum albumin (Rho: -0.68, *P*<0.01) (Figure 3), and number of sclerotic glomeruli (Rho= -0.33, *P*=0.022), although the latter correlation was not considered in the adjusted model because it was an effect of the independent variable.

The subgroup with a lower proteinuria/BMI ratio had a male prevalence (43% vs 19%, *P*=0.06), and interestingly higher albumin concentration (3.0±0.7 g/dl vs 2.2±0.6 g/dl, *P*=0.001).

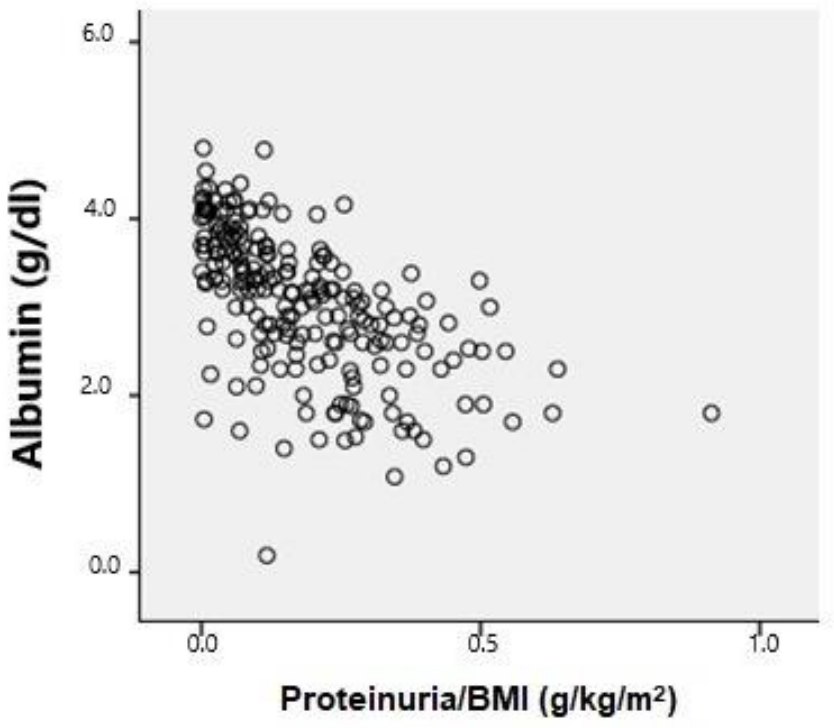

Figure 3. Scattergraph reporting distribution of albumin (g/dl) and proteinuria/BMI (g/kg/m^2).

The unadjusted analysis showed a negative association between time*proteinuria/BMI ratio and eGFR (β:-9.05, *P*=0.07, 95% CI:-18.85/-0.76), confirmed in the adjusted model (β:-11.57, *P*=0.04, 95% CI=-22.96/-0.19), as reported in table 3.

Dependent variable: eGFR (CKD-EPI formula)						
	Unadjusted model			**Adjusted model 1**		
	Coefficient	***P***	**95% IC**	**Coefficient**	***P***	**95% IC**
Time	-2.76	0.03	-5.22/-0.29	-2.51	0.08	-5.29/0.26
Proteinuria/BMI ratio	18.04	0.2	-9.53/45.6	13.51	0.04	-16.78/46.79
Time* proteinuria/BMI ratio	**-9.05**	**0.07**	**-18.9/0.76**	**-11.6**	**0.04**	**-22.96/-0.19**
Age (years)				**-0.99**	**0.001**	**-1.29/-0.70**
Sex (M/F)				8.00	0.15	-2.89/18.89
Arterial hypertension (no/yes)				5.40	0.40	-7.24/18.04
Diabetes mellitus (no/yes)				-3.50	0.70	-21.25/14.25
Albumin (g/dl)				-1.07	0.57	-4.76/2.62
Use of rituximab (no/yes)				-4.45	0.43	-15.57/6.66
Conservative treatment				**-8.52**	**0.06**	**-17.43/0.38**

Table 3 Adjusted model according to eGFR as dependent variable

Age was related to a reduction of eGFR (adjβ:-0.99, 95%CI: -1.29/-0.70, *P*=0.001), as well as the conservative treatment compared to Ponticelli regimen (adjβ:-8.52, 95%CI -17.43/0.38, *P*=0.06). No differences were detected either in the adoption of rituximab or Ponticelli, nor between other variables and eGFR.

Linear combination was performed to detect the impact of the time as effect modifier (figure 4).

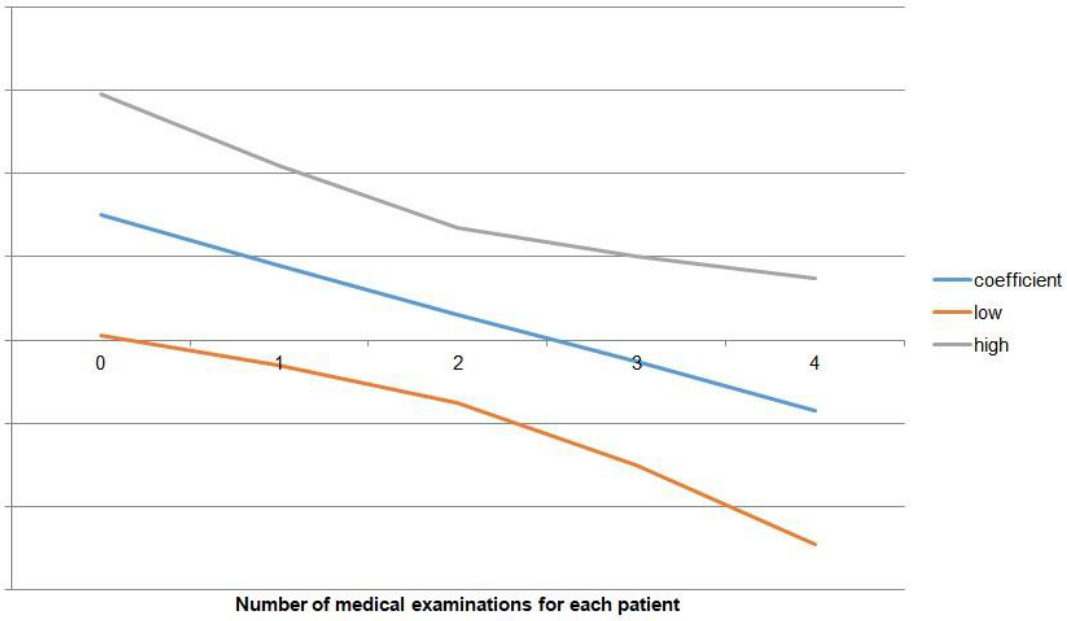

Figure 4 Effects of time (expressed as the number of medical examinations for each patient) modulating the impact of proteinuria/BMI (g/kg/m^2) on eGFR (ml/min/1.73 m^2) .

5. Discussion

Our study analyzed the longitudinal impact of BMI on residual kidney function in patients affected by MN and with the same amount of proteinuria. In this analysis, the proteinuria/BMI ratio was positively related to eGFR, but the trend changed according to time. Conversely to conservative treatment with renin-angiotensin-aldosterone system inhibitors (RAASi) compared to the glucocorticoids and cyclophosphamide (Ponticelli regimen), the choice of rituximab or Ponticelli scheme did not differ significantly in terms of disease progression of the patients.

In our analysis, the time was an effect modifier reducing the eGFR. Its impact could be easily explained: although the proteinuria/BMI ratio was directly related to eGFR, the time between the follow-up examination also marked the increased age, which is known to inversely affect eGFR. A slope of about 1 ml/min/1.73 m^2 of eGFR happened each year, according to the physiological ageing, with the consequent impact on the progression over time.

The study conducted by Xiaofan et al confirmed the correlation between age and the adverse outcome of the progression of kidney disease, ESRD and death in the Asian population by using eGFR by CKD-EPI equation as a marker of kidney function instead of serum creatinine[30]. The same study also shows that older patients with lower eGFR and severe proteinuria at the moment of renal biopsy were increasingly exposed to adverse outcomes[30].

Several studies have reported associations between serum creatinine, proteinuria and reduction of eGFR in patients with MN. The excretion of low molecular weight proteins as an alternative to 24-hour proteinuria does not improve the prediction of long-term outcomes or response to the Ponticelli regimen, although they are predictive of a 12-month rituximab response[31].

BMI was already reported in previous studies as a risk factor, as by Russo and coll[32] who performed a longitudinal evaluation of its impact on the risk of dialysis initiation in CKD patients. BMI was considered a risk factor if compared to the residual renal function. In our analysis, indeed, higher BMI for the same degree of proteinuria was associated with a lower proteinuria/BMI ratio, linked to a worse progression over time. We did not stratify patients according to normal weight or obesity classes as defined by the World Health Organization (WHO) (table 4), but BMI was included as a continuous variable, with a mean value of about 26 kg/m^2, which was above the normality range (18,5-24,9 kg/m^2) and corresponded to overweight class.

Weight	Body Mass Index (kg/m^2)
Underweight	< 18.5 kg/m^2
Normal range	18. 5 – 24.9 kg/m^2
Overweight	25 – 29.9 kg/m^2
Obesity class I	30.0 – 34.9 kg/m^2
Obesity class II	35 – 39.9 kg/m^2
Obesity class III	≥ 40 kg/m^2

Table 4. World Health Organization classification of weight status according to Body Mass Index.

The effect of BMI on CKD is well known although there was no evidence of the relationship between BMI and clinical-pathologic parameters in MN[33]. In detail, Cai et al reported a 23% increase in the risk of moderate CKD for each standard deviation (SD) from normal to higher rates of BMI (OR = 1.230, P = 0.017) in a sample of 7953 enrolled patients[34].

The impact of obesity was evaluated in the Cure GN cohort including 1,548 adults affected by glomerular diseases. Despite a higher incidence of primary kidney endpoint (40% eGFR decline or ESRD) in the class II-III obesity group vs normal weight group (90.8 per 1,000 person-years vs 58.0 per 1,000 p-y), in multivariate-adjusted analysis, this finding did not remain significant. Higher BMI was rather confirmed as a risk factor for cardiovascular events[35].

Yonekura et al evaluated the role of BMI on kidney disease progression in a large cross-sectional Japan registry analysis[36]. In particular, the authors reported that proteinuria was higher in patients with larger physical constitution, defined by both BMI >25 kg/m^2 and body surface area (BSA) >1.73 m^2, compared to those with normal/low physical constitution. In a subgroup of patients affected by MN (n=696), average proteinuria was 6.08 ± 3.19 g/day in the low-BMI group versus 7.23 ± 3.97 g/day in the high-BMI group (*P*=0.003). by

From a pathophysiological point of view, it is believed that the increase in fat mass causes mesangial expansion[37] and elevates renal metabolic demand, promoting glomerular hyperfiltration, hypertrophy and the decrease in density of podocytes, which increases the filtration fraction[38-40]. This hypothesis was confirmed by Chen X et al, in a cohort of MN patients where BMI was related to the extent of mesangial lesions[33]. Despite this observational study did not evaluate longitudinally the progression of renal disease, it posed the hypothesis of increased chronic damage load on glomeruli due to higher BMI values.

Moreover, dyslipidemia, whether secondary to nephrotic syndrome or enhanced by obesity and metabolic syndrome, results in lipid accumulation in kidney tubules, prompting inflammation and oxidative stress in a process known as lipotoxicity. This stimulates tubular epithelial cell apoptosis and progressive tubulointerstitial fibrosis up to tubular atrophy[41,42]. In particular, Guan et al demonstrated in 363 MN patients that the disbalance between triglycerides and HDL-c, expressed as TG/HDL-c ratio, had a non-linear correlation with tubular atrophy, with an inflection point at a value of 4.25[43].

Together all these processes can collectively contribute to the development and progression of CKD, triggering downstream activation of pathways such as the renin-angiotensin-aldosterone and TGF-beta systems. Transcriptomics also reports structural changes in glomeruli in obese patients. A small study in adults with obesity-related glomerulopathy found that there was a different expression of genes involved in regulating lipid metabolism, inflammation and insulin response, which contribute actively to the pathogenesis of glomerular damage[44].

Beyond these pathophysiological explanation, BMI is related to variations in drug bioavailability and metabolism too, modulating the effect of the immunosuppressive treatment. According to this hypothesis, Goleva et al[6] and Sawamoto K et al[45] reported the effects of obesity on glucocorticoids and tacrolimus' distribution and metabolism, respectively.

Obese adults have an enhanced activation of type 1 5α-reductase in the liver, as evidenced by increased excretions of cortisol as 5α-reduced metabolites compared to normal-weight adults[12]. It is plausible that a higher metabolic clearance rate may lead to reduced drug exposition, resulting in lower chances of achieving complete remission, similar to what has been reported in MN with CNI[12].

However, the adoption of BMI as a parameter for obesity in edematous subjects is problematic, since it does not take into account volume overload; practical measures should be taken to distinguish weight gain from sodium retention and true adiposity.

As regards therapeutic management of MN, it is mainly related to the risk of progressive loss of kidney function according to the 2021 KDIGO Guidelines[46].

Patients are treated with supportive treatment such as RAASi if low-risk, while the adoption of rituximab, glucocorticoids in association to cyclophosphamide or CNI or other immunosuppressors such as mycophenolate is reserved to patients with moderate to high risk of progression to ESRD, in a case-by-case evaluation[12].

In our analysis, patients treated exclusively with optimized conservative approach (RAASi at maximum tolerated dose) experienced a higher eGFR slope during follow-up compared to patients treated with Ponticelli regimen, despite being classified as low-risk. Conversely, no differences in efficacy were found between Ponticelli regimen and rituximab, as it is actually stated by literature[47,48]. To explain this findings, we analyzed the proteinuria/BMI ratio in the three group of patients undergoing conservative treatment, cyclical combination of glucocorticoids and cyclophosphamide, and rituximab. Patients with supportive therapy had a lower proteinuria/BMI ratio than the other two groups (figure 5), without significant differences in BMI. This result allows us to speculate that the amount of proteinuria should also be related to BMI to better stratify the risk of disease progression.

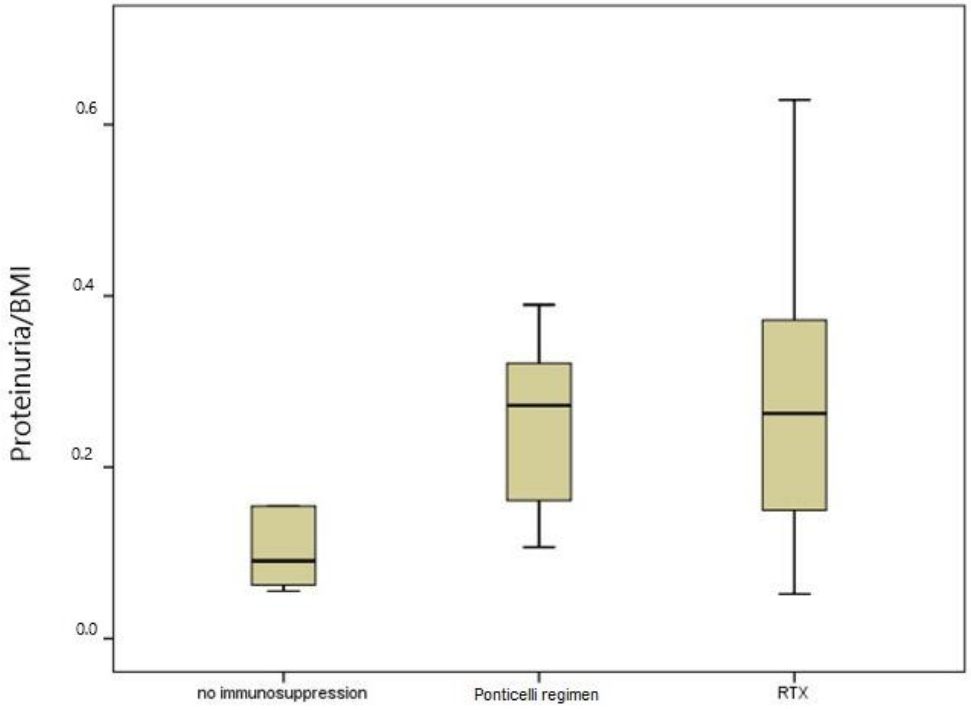

Figure 5 Distribution of the ratio proteinuria/BMI in treatment-based subgroups of patients.

6. Conclusions

The observational design is the major limit of our study, and missing data concerning the treatment adherence as well as the adverse events could be considered other limitations. As regards these aspects, Chen et al reported in their recent meta-analysis the efficacy and the adverse events of alternative treatments to Ponticelli regimen, showing that rituximab had less prevalence of bone marrow suppression without affecting the remission rate, independently from the baseline proteinuria[49].

Despite these limitations, the longitudinal evaluation of a cohort of 61 patients affected by MN should be considered the strong point of our analysis, because few similar evaluations have been performed pointing out the impact of BMI on eGFR changes in patients affected by a rare disease.

In conclusion, although larger studies are needed and should be encouraged to confirm our results, we can speculate that the proteinuria/BMI ratio, which can be considered as the proteinuria for each unit of BMI, impairs *per se* renal outcomes, as well as higher BMI levels up to obesity are associated with elevated risk for renal function loss over time when longitudinally evaluated.

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