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Membranous Nephropathy: Anatomo-Clinical characterization, Therapeutic Intervention and Outcomes

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Do coração, bem fundo

Digo-vos em plena voz

Porque sois o meu Mundo

Por isso, o devo a vós.

RESUMO:

Introdução: A Nefropatia Membranosa lidera as causas de síndrome nefrótico nos adultos. As suas decisões terapêuticas são atualmente determinadas por "*scores*" clínicos, laboratoriais e histológicos. A inclusão do anticorpo do receptor da fosfolipase A2 nessa abordagem pode representar uma mudança de paradigma**.**

Objetivos: O nosso grupo teve como objetivos 1. caracterizar fenótipos da nefropatia (clínicos, histológicos e analíticos) 2. definir progressão e respostas terapêuticas na população analisada 3. avaliar resultados, identificando preditores de sobrevivência renal e de trajetória de proteinúria.

Métodos: Foram analisados registos eletrónicos de indivíduos diagnosticados com nefropatia membranosa (n = 32). Definiu-se a linha de base do estudo como a data da biópsia-índice. As biópsias foram classificadas de acordo com parâmetros histológicos. Variáveis clínicas e analíticas foram seguidas regularmente (mês 3, 6, 12 e anualmente) até maio / 2019 (tempo de seguimento médio de 3 anos e 6 meses). Os preditores da trajetória foram avaliados por modelos de regressão mista linear uni e multivariada. Foram exploradas as curvas de sobrevivência e preditores do resultado clínico composto (início de diálise ou redução > 50% de taxa de filtração glomerular).

Resultados: A fibrose intersticial e a atrofia tubular associaram-se a aumento da creatinina sérica (+0,425 mg / dL por nível histológico) e à diminuição da taxa de filtração glomerular (-38,9% / nível). A hipercelularidade mesangial foi associada ao aumento da proteinúria anual (+ 0,4 g / g por nível; P = 0,002). Idade ≥ 60 anos (-1,55 vs -0,96 g / g; P = 0,006), sexo feminino (-1,5 vs -1,06g / g; P = 0,044) e creatinina basal <1mg / dL (-1,3 vs -0,54 g / g; P = 0,001) foram associados a maiores descidas na proteinúria anual. Remissões parciais/completas apresentaram melhor sobrevida renal que casos sem remissão (p = 0,001). A creatinina basal ≥1,00mg / dL (risco 13,75x maior) e a presença de remissão (0,040x menor) foram identificadas como variáveis preditoras independentes do resultado clínico composto (segundo o modelo de regressão multivariável de Cox ajustado para idade, sexo e imunossupressão).

Conclusões: Sexo, idade, creatinina basal e "*scores*" histológicas associaram-se à trajetória da proteinúria. A creatinina e a remissão basais foram associaram-se de forma independente à sobrevida renal. A inclusão do anticorpo para o antigénio do recetor da fosfolipase A2 no modelo preditivo poderá melhorar a individualização da terapia e a previsão do prognóstico.

Palavras-Chave:

Glomerulopatia; Membranosa; Nefropatia Membranosa; Nefrótico; Síndrome Nefrótico

ABSTRACT

Background: Membranous nephropathy leads the causes for adult nephrotic syndrome. Clinical, laboratory and histologic scores determine therapeutic decisions. Including phospholipase A2 receptor antigen in this approach can be paradigm changing. Our group aimed to 1. characterize this nephropathy's phenotypes (clinical, histological, analytical) 2. define progression and treatment responses in surveyed population 3. evaluate outcomes, identifying renal survival and proteinuria trajectory predictors.

Methods: Electronic records of diagnosed cases (n= 32) were analysed. Index biopsy date defined the study baseline. Biopsies were scored according to histological parameters. Clinical and analytical variables were followed regularly (month 3, 6, 12 and annually) until May/2019 (42 months median follow-up). Annual-proteinuria-change predictors were assessed by univariate and multivariable linear mixed regressions. Survival curves and predictors of the composite outcome (dialysis start or >50% decreased glomerular filtration rate) were explored.

Results: Interstitial fibrosis and tubular atrophy were linked to serum creatinine (+0.425 mg/dL per histological level) and glomerular filtration rate (-38,9%/level). Mesangial hypercellularity was associated with increased annual proteinuria (+0.4g/g per level; P=0.002). Age ≥ 60 years (-1.55 vs -0.96 g/g; P=0.006), female gender (-1.5 vs -1.06g/g; P=0.044) and baseline creatinine<1mg/dL (-1.3 vs -0.54 g/g; P=0.001) were associated with greater descents in annual proteinuria. Partial/complete remissions displayed better survival than non-remitting cases(p=0.001). Baseline creatinine ≥1.00mg/dL (13.75x higher risk) and presence of remission (0.040x lower) were independently associated to the composite outcome (multivariable Cox Regression Model adjusted for age, gender and immunosuppression).

Conclusions: Sex, age, baseline creatinine and histologic score were associated with proteinuria trajectory. Baseline creatinine and remission were independently associated with renal survival. Inclusion of phospholipase A2 receptor antigen in the predictive model will hopefully improve therapy individualization and prognosis prediction.

Key-Words:

Glomerulopathy; Membranous; Membranous Nephropathy; Nephrotic; Nephrotic Syndrome

LIST OF ABBREVIATIONS

ACEi – Angiotensin-Converting Enzyme inhibitor

AKI – Acute Kidney Injury

ANCA – anti-neutrophil cytoplasmic antibody

Anti- dsDNA – Double-stranded DNA antibodies

Anti- Fx1A – Double-stranded DNA antibodies

ARB – Angiotensin II Receptor Blocker

C – Complement

CHUP – Centro Hospitalar da Universidade do Porto

CKD – Chronic Kidney Disease

CNI – Calcineurin Inhibitors

CR – Complete Remission

eGFR – estimated Glomerular Filtration Rate

EM – electron microscopy

ESRD – End-Stage Renal Disease

FSGS – Focal Segmentar Glomerulosclerosis

GBM – Glomerular Basement Membrane

GN – Glomerulonephritis

GvHD – Graft vs Host Disease

HBV – Hepatitis-B Virus

HCV – Hepatitis C Virus

HIV – Human Immunodeficiency Virus

IC – Immune Complexes

IF – Immunofluorescence

Ig –Immunoglobulin

IHC - Immunohistochemical

IST – Immunossupressive Treatment

MAC – Membrane Attack Complex (complement-mediated)

MBL – mannose-binding Lectin

MCD – Minimal Change Disease

MN – Membranous Nephropathy

NELL-1 – Neural epidermal growth factor-like 1 protein

NEP - Neuroendopeptidase

NS – Nephrotic Syndrome

NSAID – Non-Steroid Anti-Inflammatory Drug

PGN – Primary Glomerulonephritis

PLA_2R1 - Phospholipase-A2-Receptor

PMN – Primary Membranous Nephropathy

PR – Partial Remission

RA – Rheumatoid Arthritis

RTX – Rituximab

SC – supportive care

Anti-Sm – anti-Smith antibodies

SMN – Secondary Membranous Nephropathy

$sPLA_2R1$ - soluble Phospholipase-A2

SR – Spontaneous Remission

THSD7A – Thrombospondin type 1 domain-containing 7A

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INTRODUCTION

Membranous nephropathy (MN) is an immune-mediated glomerular disease and the leading cause of adult nephrotic syndrome (NS)[1]. One third of patients enter spontaneous remission (SR) in the first 2 years and 15%-40% of the patients reach end-stage renal disease (ESRD) in 5-15 years (even if under immunosuppressive treatment (IST)) [2,3]. Therefore, it is the 2nd cause of ESRD among primary glomerulonephritis (PGN)[4]. MN is characterized by glomerular basement membrane (GBM) alterations, due to glomerular sub-epithelial immune complex (IC)[5]deposition[6], among other GBM injury factors (complement activation, genetic predisposition and environmental "triggers"). Recent progresses improved our understanding of MN as a disease and phospholipase-A2-receptor (PLA_2R1)-antibody serum titles and their correlations are being focused in the investigation of this glomerulonephritis. In Portugal, we lack a registry-based study to characterise the entity and promote new evaluation algorithms.

This study aims to identify adult patients diagnosed with MN in the population surveyed by Centro Hospitalar Universitário do Porto (CHUP), in order to 1. characterize clinical, histological and analytical phenotypes of this glomerulonephritis, 2. define progression profiles and response to the therapeutic interventions, 3. evaluate the outcomes of our actual approach and identify predictive variables of proteinuria trajectories and renal survival.

Epidemiology and Classification

MN accounts for 20-37% of total NS in adults[7]. Its annual incidence ranges from 0.2/100.000 to 1.4/100.000 individuals[8–10], with a 2:1 male predominance[11,12]. MN mostly affects middle-aged and older individuals [10,11] specially Caucasians (with an annual incidence of 1.7/100.000[13]).

MN cases can be subdivided in primary MN (PMN), accounting for 70-80% of total MN [7,14] and secondary MN (SMN). Secondary MN (SMN) can be caused by systemic or infectious diseases (sarcoidosis, HIV, HBV or HCV[15]), malignancy (lung, prostate, hematologic, melanoma[16]), auto-immune or alloimmune diseases (systemic lupus erythematosus, thyroiditis, rheumatoid arthritis, graft vs host disease or *de novo* MN in transplants [17]) and drugs/toxin exposure (non-steroid anti-Inflammatory drugs, d-penicillamine, captopril, mercury, formaldehyde and air pollutants[18,19]).

Clinical Presentation and Laboratory Exams

MN typically presents with NS (proteinuria ≥3.5g/day, hyperlipidaemia, peripheral oedemas and hypoalbuminemia) in 60-80%[3,4]of cases. 60% of the initially non-nephrotic will progress to NS[20]. Decreased estimated glomerular filtration rate (eGFR) is present initially in 10-20%[7], reaching 20-40% along the follow-up[1]. Microscopic haematuria is initially present in 50%[20] and arterial hypertension (associated with a worse prognosis, disease progression and decreased eGFR[9]) in 25-30%[21] of MN cases.

MN serological presentation is variable. In SMN, serologies will display aspects relative to its etiology (e.g. in lupus nephritis related-MN, anti-nuclear (ANA), anti-dsDNA and anti-Sm antibodies should be increased). PMN can present as PLA_2R1, thrombospondin type 1 domain-containing 7A (THSD7A) or neural epidermal growth factor-like 1 protein (NELL-1) antibody-positive or serum negative. No patient positive for two of these antibodies has been found[4,22,23]. Some SMN can be PLA_2R1[24] or THSD7A[16] antibody-positive. Therefore, our approach to serological results must be pondered and careful, in order not to mislabel or misdiagnose MN patients.

PLA_2R1 antibodies (and its titers) were also associated with specific clinical and laboratorial presentations: patients with higher titers displayed higher baseline proteinuria [25], increased baseline serum creatinine and decreased eGFR[26].

Histopathology

Light microscopy imaging of precocious MN, can reveal absolutely no alterations[7], or detect the typical sub-epithelial deposits. Ehrenreich created a MN histological classification scale (using light microscopy imaging), dividing it in 4 stages: stage I (sub-epithelial deposits, with/without slight thickening of GBM), stage II ("spiked" deposits protruding from the GBM), stage III (deposit inclusion within a thickened GBM) and stage IV (irregularly thickened GBM at its maximum width, with some partially reabsorbed deposits). Complete remissions (CR) display a stage IV with complete reabsorption of some deposits, leaving lucent areas in the GBM (ultimately regressing to a normal state). [27] Despite all that, other imaging techniques are required to confirm MN's diagnosis.

Immunofluorescence (IF) should detect granular sub-epithelial deposits, uniformly distributed in the glomeruli, staining predominantly with IgG4 (or IgG1 and 3 in early PMN[28]) antibodies directed to the PLA_2R1 antigen. They can also detect staining for complement system's membrane attack complex (MAC), colocalized with those antibodies (as the complement system is central in

PMN)[27]. SMN can reveal coexisting subepithelial and mesangial deposits, staining for IgG1,IgG3, IgM and IgA, C1q,C3 and C4 ("full-house" immunostaining)[27]. Detection of these last IF patterns should prompt and adequate search for causes of SMN.

Definitive diagnosis of MN requires an electronic microscopy (EM) image showing discontinuous electron-dense sub-epithelial IC deposits (in the outer surface of the glomerular capillary wall, matching the C3 and IgG4 deposits seen in IF) and effacement of glomerular podocytes. Subepithelial or mesangial deposits should raise the suspicion for SMN, as glomerular reticular aggregates[27].

Pathophysiology and biomarkers of disease

In 1946, Bell characterized MN as a glomerulonephritis with a thickened GBM, associated with marked proteinuria and oedema. In 1959, Movat linked the GBM thickening with a sub-epithelial Ig deposition (and IC formation), between the GBM and podocytes[29]. Couser and Hoedemaker proved that IC seen in biopsies were developed *in situ* (by direct binding of the autoantibodies to the podocyte antigens)[30,31].

In 2002, the first human MN-associated antigen was found. Neuroendopeptidase (NEP) (present in the podocytes and brush border of renal tubules) was determined as causal factor for neonatal MN. The NEP-positive neonates, born from NEP-negative mothers, displayed renal biopsy and clinical features of MN, after their mothers developed an immune response to NEP, based on NEP-antibody production (transported through the placenta)[32].

In 2009 Beck discovered PLA_2R1 and its antibody (present in 70-80% of all PMN)[33]. PLA_2R1 is a superficial transmembrane glycoprotein receptor. M-type PLA_2R1 is a receptor for soluble Phospholipase-A2 ($sPLA_2$), an inflammatory mediator, whose function is yet to be known (*in vitro*, it mediates inflammatory and pro-apoptotic processes[34], but some believe that it reduces $sPLA_2$'s inflammatory effects[4]). PLA_2R1-antibodies, are predominantly IgG (mostly IgG4[33]) highly specific for the antigen (most likely being in the origin of podocyte injury and proteinuria). They were also located in PMN kidney biopsies, colocalized with their antigen, within the subepithelial deposits. PLA_2R1 antibodies were only found in serum and/or kidney biopsies of patients with PMN [33]. Antibody titers were correlated with disease activity: patients with higher proteinuria and/or lower serum albumin presented with higher serum titles of PLA_2R1-Antibody[33].

Human PLA_2R1 is also present in neutrophils, pulmonary macrophages, airway epithelial and submucosal epithelial cells[35,36]. Damage to any cells containing PLA_2R1 can induce the release of

vesicles with the antigen. Through complex interactions between genetic predisposing factors and a pro-inflammatory environment (e.g increased levels of air pollution[18]), the exposure of PLA_2R1 can create a self-directed immunological response, mediated by Ig's and the complement system, damaging the filtration barrier, creating PMN's typical histological specificities and clinically-detectable proteinuria.[5]

In 2014, serum samples from 2 cohorts with patients initially labelled as "PLA_2R1-antibody negative PMN" were analysed. 10% were serum-positive for THSD7A-antibody (representing around 3% of MN). No PLA_2R1-antibody-positive patient was positive for THSD7A (suggesting they represent different entities). The THSD7A-antibodies were predominantly IgG4 and were linearly dispersed in the subepithelial deposits (using immunohistochemical techniques) along the GBM. They were colocalized (using IF) with their specific THSD7A antigen, in kidney biopsy samples (indicative of their role in MN's physiopathology). An association was suggested between disease activity and serum antibody titers, as relapses were preceded by an increase in serum titers and clinical response to IST was associated with decreased titers.[22] THSD7A-antbody positive patients were more often female (65% vs 27%)[22] and younger[37], than PLA_2R1-antibody positive patients. This antibody was also associated with some cases of SMN, specifically malignancy-associated) [16].

Finally, in 2020, the latest PMN antigen, NELL-1, was discovered. "PLA_2R1-antibody negative PMN" patient biopsies were identified. Using mass spectrometry techniques, an antigen with a similar expression pattern to PLA_2R1 was identified, called NELL-1 (in 16% of PLA_2R1-antibody negative PMN, around 5% of PMN patients). No PLA_2R1-antibody positive PMN was NELL-1 positive (suggesting they represent different entities). NELL-1 antibody positive PMN's were older and more likely to present with NS than PLA_2R1-positive ones. NELL-1- antibodies show a granular staining for this antigen along the GBM, colocalized (using IF) with its respective IgG, within the typical subepithelial deposits (indicative of its role in MN's pathophysiology). Antibody-titers have not been correlated with disease activity [23].

Complement system's role in MN's pathogenesis has also been investigated: when compared to healthy rats, C3-defficient rats display a greater delay between exposure to anti-Fx1A antibody (an antibody known to induce MN in rats[30]) and proteinuria[38], as did C6/C8-defficient rats[39,40]; MN renal biopsies and urinary samples show increased levels of C5b-C9, proportional to MN's severity and linked with worse prognosis[41]. These findings suggest that complement has a main role in MN's pathogenesis. The lack of C1q in PMN renal biopsies reveals that the complement pathways primarily involved in PMN are: the alternative pathway and the lectin pathway[42].

Mutations in specific genetic loci, like the human leukocyte antigen (HLA)-DQA1 (in chromosome 6p21) and the one coding for PLA_2R1(chromosome 2q24), increase PMN risk[43,44]. HLA-DQA1 codes for the major histocompatibility complex receptor class II. Its mutation can shape it to better fit PLA_2R1. PLA_2R1 mutations can alter its enzymatic fragmentation, increasing circulating levels of PLA_2R1, promoting its exposure to the immune system, and a directed immune response[45] Patients with both loci mutated have 80x higher risk for PMN than general popuplation[44]. Despite that, these mutations are common in the general population and have been found not to be the sole contributor to MN's origin, which requires a combination of genetic susceptibility factors with environmental triggers, yet to be known.

The following 3 paragraphs sum up the most commonly agreed model for MN pathogenesis.

In an early stage, through a complex interaction between genetic factors and a pro-inflammatory environment, plasma cells create PLA_2R1-antibodies that attach to their kidney antigens, in an increasing fashion. At some point, they become detectable in kidney biopsies (IF and EM).

An immunologic response towards the antigen is created, glomeruli are injured and proteinuria increases. Serum samples should still be negative, as the kidney acts "*as a sink*", retaining all glomerular antibodies[46]. Antibody production keeps rising until they become detectable in serum samples (at that point, PMN becomes serum-positive).

In remissions, antibody production decreases. The patients become (initially) serum-negative but are still "biopsy-positive" for the antibodies (maintaining the immune response and associated proteinuria). As the antibodies gradually unbind from their antigens, the kidney initiates its regeneration and proteinuria decreases. In complete remission (CR), serum samples and kidney biopsies both will be "*antibody-negative*"[14].

This sequence of events creates 2 major "*delays*": one between when biopsy becomes antibody positive and serological positivity, and one between serological/clinical remission and biopsy-remission.[47,48]

Diagnosis and Follow-Up

All cases suspect for MN must have a thorough exploration of history and physical examination (to differentiate PMN from SMN, detect clinical signs of NS, elevated blood pressure or NS complications such as renal vein thrombosis), accompanied by proteinuria (initially with 24-h proteinuria, to increase accuracy of the evaluation[49]), eGFR (preferably using chronic kidney

disease epidemiology collaboration's – CKD-EPI – equation[50]) and serum albumin assessments. These help to determine prognosis, risk of complications, correctly diagnose and classify patients with MN (allowing an individualized treatment approach). Initial haematuria and serum lipid levels (to detect the typical hyperlipidemia of NS) should be evaluated. SMN causes should be rule out (HIV, syphilis, HBV and HCV infections, anti-nuclear antibodies, anti-dsDNA, ANCA, anti-GBM, rheumatoid factor, C3 and C4 levels. Based on age, gender and individual risk factors, malignancies must be ruled out, with a basic generic screening involving thoracic x-ray (or CT, in cases of high risk for lung malignancy[51]). MN's definitive diagnosis remains, currently, mainly histological (as explained before). However, these techniques may not be sufficient to differentiate PMN and SMN (the absence of an obvious cause does not exclude SMN, and the presence of a possible cause does not mean that this MN originates from such cause, as it will be further explained). Integration of new diagnostic approaches are, therefore, in order.

Beck's 2009 study showed that PLA_2R1-antibodies display a 100% specificity and 70% sensitivity[33]. Following series showed that PLA_2R1 expression in PMN patients vary between age group (<5% of children with PMN present PLA_2R1-antibodies in their serum[52]) and region (Asian PMN cohorts only express PLA_2R1-antibodies in 50-70%[53,54]). After Beck's study, some PLA_2R1-atibody positive SMN were found (associated with hepatitis B virus infections[24], malignancies, lupus nephritis[55] and sarcoidosis[56]). Experts assume they are cases of coincident PMN and the other disease[57]. A 2014 meta-analysis demonstrated a 99% specificity of PLA_2R1-antibodies, as diagnostic markers for PMN[58].

Following these findings, some experts believe in the possibility of an initially serological approach (to detect and measure PLA_2R1-antibodies), replacing biopsy as the first procedure (when investigating an MN suspected case). If the patient is PLA_2R1-negative, kidney biopsy should be performed. If it is positive, the patient is labelled as "PLA_2R1-positive PMN" and biopsy is redundant. After diagnosing MN, the patient should be evaluated in terms of his/her progression to ESRD risk and each patient should be approached according to his specific treatment orientations.[59]

Patients should have regular check-ups every 1-12 months (time intervals variate according to clinical status, remission, comorbidities, therapeutic options and laboratorial results) re-taking patients' clinical history, physical examination (evaluating clinical aspects of the disease, therapy and their complications) and evaluating laboratory results (proteinuria, eGFR, serum albumin, serum drug levels, serum lipid screening, individual serological markers).

Higher antibody levels were associated with more serious disease, increased proteinuria, decreased serum albumin (and decreased likelihood of remission) [60,61]. This creates an opportunity to use this antibody to monitor treatment response and/or clinical state. The previously mentioned delay from serological to histological remission must always be considered.

It is yet unclear to what extent a decrease in this antibody's serum levels predicts a subsequent remission[59]. In spite of that, it may become useful in the presence of clinical worsening or increased proteinuria, working as a differentiator between a secondary AKI factor or relapse[64,73].

Treatment

Treatment for MN is based on the consensus guidelines reached in the 2012 in the "Kidney Disease: Improving Global Outcomes (KDIGO)" conference[12]. All MN patients should receive supportive care (SC). This includes blood pressure control (daily sodium intake <1,5g, exercise, smoking cessation and antihypertensive drugs), administering angiotensin-converting enzyme inhibitors/angiotensin receptor blockers (ACEI/ARB) to reduce proteinuria, control blood pressure and increase the chances of remission. These patients should also control, if present, their hyperlipidemia (using statins and other lipid-lowering drugs) and oedema (salt restriction and diuretics, preferably loop diuretics[7]). SMN treatment involves SC presented and treatment directed to the suspected/diagnosed cause of MN.

Patients with hypoalbuminemia or history of an embolic event have an increased risk of subsequent embolic events, requiring treatment with low molecular weight or unfractionated heparin, followed by oral anticoagulation. Patients with hypoalbuminemia (specially ≤2.5g/dL) must ponder the benefits of anticoagulation treatment, using online personalized risk calculation methods[62].

Approaching PMN, a major decision is in order: to use IST or not. Patients with subnephrotic proteinuria have excellent prognosis and are more likely to reach SR, rendering IST useless[51]. Patients with NS or nephrotic-range proteinuria should initiate immediate IST if they present with serum creatinine >1.5md/dL, a ≥20% decrease in eGFR in the last 12 months (excluding all other causes) or if presenting NS severe complications, such as embolic events (e.g, renal vein thrombosis) or anasarca due to severe hypoalbuminemia[12]. Couser *et al* also defends the initiation of IST in patients with presenting proteinuria >10g/day or if after 3 months of SC, proteinuria levels are >8g/day[7].

After 6 months of SC, the probability of ESRD should be evaluated, according to the Toronto Risk Score[63], splitting patients in 3 classes: high risk (proteinuria >8g/day with <50% decrease in proteinuria or reduction >30% in eGFR from baseline), moderate risk (proteinuria 4-8g/day, with stable eGFR) and low risk (proteinuria <4g/day, with stable eGFR). Moderate and high-risk should initiate IST[51], if the patient does not present: echogenic kidneys <8cm in length, life-threatening infections[51]. These individuals are less responsive and suffer worse consequences of IST.

Before IST, all patients are screened for infections and age-appropriate malignancies. 3 major drug subgroups are used for IST: calcineurin inhibitors (CNI) (tacrolimus or cyclosporin), cytotoxic drugs (mainly cyclophosphamide or chlorambucil) and B-cell depletors (as rituximab (RTX)). The first two are combined with corticosteroids. KDIGO guidelines recommend kidney biopsy prior to IST, to exclude concomitant processes and estimate the extent of kidney injury[51].

Patients' treatment response must be re-evaluated, in terms of remission. KDIGO defines 3 states of remission: complete Remission (CR) (proteinuria<0.3g/day), partial remission (PR) (>50% reduction in baseline proteinuria, maintaining 0.3-3.5g/day and stable eGFR) and no remission (NR) (the remainder). "Relapse" is also defined as return of nephrotic-range proteinuria after achieving remission.

KDIGO recommends alkylating agents as first-line treatment, being the only ones proven effective in preventing ESRD or death[43]. They also have proven their long-term efficacy in time periods ≥10 years[66]. Smokers (because of increased risk of bladder and lung cancer) and women who desire children (because of increased infertility) should avoid this treatment line[51]. Patients should be administered prophylactic trimethoprim/sulfamethoxazole (to reduce the incidence of serious infections, present in up to 24% of patients in some series[65]). Other common side effects include haemorrhagic cystitis (cyclophosphamide) and bone marrow suppression (with leukopenia)[51].

There are 2 main schemes involving alkylating agents: modified Ponticelli scheme (in days 1 through 3 of months 1,3 and 5, 1g of intravenous methylprednisolone is administered, followed by oral Prednisone, 0.5mg/kg daily for 27 days; in months 2,4 and 6, 2-2.5mg/kg daily of oral cyclophosphamide should be taken) and the dutch scheme (similar, but oral prednisone is given daily for 6 months straight and 1.5-2mg/kg of cyclophosphamide is given daily for 12 months). 29% of patients enter remission after 1 year (vs 9,4% in controls) and 60-65% at 10 years (vs 35% of controls), according to various series[65,67]. It presents a 10% mortality rate and 3% reached ESRD with renal replacement therapy in 10 years[66]. After 2 years, 20% of patients relapse[65].

CNI are the second therapeutic line (used when alkylating agents are not indicated, refused or fail[51]). Daily 3.5-5mg/kg of cyclosporin is administered for 12-18 months (maintaining serum levels

about 120-200 μg/L), with 5-10mg/kg of daily oral prednisolone. Tacrolimus is similarly administered daily (0.05-0.075mg/kg, maintaining serum levels about 3-5μg/L) for 12-18 months, with the same accompanying dose of prednisolone. Remission rates can reach the 70-75% (vs 25-35% of controls)[68,69], in a shorter period than alkylating agents[70]. 47% of patients relapse in median 4 months (after treatment suspension)[68]. Common (present in 40%) adverse effects include glucose intolerance, hypertension and (transient) eGFR decrease[68,69]. CNI, especially with higher risk patients are less effective in preventing eGFR decrease. On the other hand, their side effects are less serious and their mortality rates are similar[71].

B-cell depletors, like RTX, are monoclonal antibodies used in monotherapy. Either in one 375mg/kg dose (with regular CD20 counts in follow-up) and a second dose when CD20 counts normalize, in weekly 375mg/kg doses (for 4 weeks), 1g in a single dose or 1g every 15 days (for 1 month) and another dose after 6 months. RTX presents a delayed response, as 6 months remission rates are usually comparable to patients only exposed to SC[72], but if we extend the follow-up time to 18 months, 60-80% of patients enter remission [73,74] . RTX seems to be efficient in achieving remission, especially in severe or refractory MN[75,76]. Antibiotic prophylaxis with trimethoprim/sulfametoxazole is recommended in these patients[77].

Patients with elevated pre-treatment PLA_2R1-antibody levels were less likely to enter remission (IST or non-IST) [47,78] and elevated post-treatment antibody levels were linked to increased risk of relapse.[79] The decline in circulating antibody levels tends to precede clinical remission from weeks to months[26,33]. This delay is directly proportional to serum antibody titers[80]. Resurgence of these antibodies in serum, usually precedes a clinical relapse[48].

Prognosis

One third of the patients enter SR within 15 months [2,3]. From the remainder,40-50% will progress to ESRD 5-15 years[2,3]. Besides hypertension, other factors were associated with increased likelihood of ESRD: proteinuria≥8g/day, male gender, ≥50 years of age, decreased baseline eGFR, increased serum creatinine and urinary excretion of β2-microglobulin and α1-microglobulin[3,7,60,63].

Higher PLA2R-antibody baseline serum titers were correlated with decreased likelihood of SR/PR and longer periods between treatment (IST or non-IST) and remission (PR or CR)[26,61,80]. Antibody serum titers were independently associated with remission (titers are inversely proportional to the likelihood of remission[81]) and emergence of NS in initially non-nephrotic patients (titers are

directly proportional to the likelihood of NS)[82]. PLA_2R-antibody levels were already correlated to worse renal outcomes[72,80].

MATERIAL AND METHODS

Study Population

This study was performed in CHUP's surveyed population. Individuals diagnosed with MN since 01/01/2004 until 31/12/2018 were identified from the database SAM/SClinico excluding those under 18 years (at the time of diagnosis). Their electronic records were analysed. Index biopsy date defined the study baseline. 35 individuals were initially selected. 3 of those were excluded (surveyed by a different hospital or follow-up <3months). Kidney biopsies were re-examined and classified according to the state-of-the-art. Clinical and analytical information was taken, initially and progressively (month 3, 6 and 12 and annually until 10 years of follow-up). All therapeutic decisions were analysed.

Clinical variables (birthdate, gender, history of hypertension, dyslipidaemia or diabetes mellitus, weight, height, blood pressure), treatment description and analytical results (serum creatinine, albumin and haemoglobin, proteinuria, erythrocytes and leukocytes in urine sediment, serological markers of infections with HIV, HBV, HCV, *Treponema pallidum*, serologic levels of C3, C4, IgM, IgA, IgG, ANA's, ANCA's, anti-GBM, anti-PLA_2R1) were recorded initially and in the previously mentioned intervals (if applicable).
The following renal histology variables were evaluated: Hypercellularity (HC) either mesangial or endocapillar, Interstitial Fibrosis and Tubular Atrophy (IFTA), IF staining for IgA (IFIgA), IgG (IFIgG), IgM (IFIgM), C3 (IFC3) and C4 (IFC4). Those variables were correlated with laboratorial values.

Patients were classified according to their remission status after 2 years, following KDIGO guidelines[51]in: complete remission (CR, proteinuria<0.3g/g creatinine with stable estimated glomerular filtration rate (eGFR)), partial remission (PR, present proteinuria <3.5g/g creatinine, with a >50% reduction in proteinuria), and no remission (NR, the remainder). Spontaneous Remission (SR) was considerered when proteinuria decreased to <0.3g/day without IST.

Statistical Analysis

Continuous data was described using median (interquartile range [IQR]), and categorical data was expressed as number (and percentages). Categorical data was compared using Pearson $\chi 2$ test or

Fisher exact test, and continuous variables were compared with Mann-Whitney U or Kruskal-Wallis test, as appropriate. Correlation between histological features grading and laboratory and clinical data was determined by Spearman correlation.

Annual proteinuria change in the first 2-years was assessed by univariate and multivariable linear mixed regression model, that imputed subject-specific random effects (intercept and slope defined as initial proteinuria and time in years, respectively) on an unstructured covariance matrix. The dependent variable was all proteinuria measurements (median 5, IQR: 5-5) and the independent variables were entered as 2-way interaction terms between themselves and the time (in years) variable. Only variables with a univariate P-value <0.150 were included in the multivariable model.

Survival curves for the defined composite outcome (dialysis start or reduction in eGFR >50%) were constructed using Kaplan-Meier method and between groups comparison was done by log-rank test. Lack of such composite outcome was determined as “renal survival”. Independent predictors of the composite outcome were detected by Cox proportional hazards regression.

A 2-sided P-value<0.05 was considered as statistically significant. Statistical calculations were performed using STATA/MP, version 15.1 (Stata Corp, College Station, TX).

RESULTS

Baseline Analysis

The 32 MN patients included 13 women and 19 men, with median 61.8 years of age (IQR 44.2-68.7) and 72.5kg body weight (IQR 63.5 –84.3). 23 presented with NS (72%), 21 with dyslipidaemia (66%), 4 diabetes (13%) and 20 individuals were hypertensive (63%). The latter were medicated with median 1 antihypertensive (IQR 0-2) and 14 patients (44%) included an ACEi or an ARA in their regular medication. Median follow-up time was 3.5 years (1.9-6.3) (Table I).

Baseline serum creatinine median levels was 0.97mg/dL (0.74-1.41), reflecting a median eGFR of 80.8mL/min/1.73m^2 (44.3-109). 14 people (44%) presented decreased eGFR (<60ml/min/1.73m^2). 16 patients (50%) presented with haematuria, median serum albumin was 2.52 mg/dL (2.12-3.1) and median proteinuria was 5.8 g/g of creatinine (IQR 4.3-8.1) (Table I).

At the baseline, we divided the patients in IST and non-IST. The IST group was significantly (p=0.017) younger (50.6 years, IQR 43-64.6 versus 68.6 years, IQR 63.6-73.4) and more likely to present with NS than the non-IST (86% vs 40%, respectively, with p=0.013). There was no

significant baseline difference in gender, follow-up time, body weight, serum creatinine, eGFR, proteinuria, history of arterial hypertension or diabetes, anti-hypertensive drugs (number and type) or dyslipidaemia medication between the 2 groups. (Table I)

Since PLA_2R1-antibody detection in serum samples was only initiated in 2016, only 6 patients with suspected PMN had their serums tested. 4 of those had positive serum samples (66,6%).

Renal Biopsy Results and Correlations

A median 12 glomeruli (IQR 7-18) per biopsy (n=32) were analysed. The following variables were evaluated: Hypercellularity (HC) in the mesangial and endocapillary regions, Interstitial Fibrosis and Tubular Atrophy (IFTA), IF staining for IgA (IFIgA), IgG (IFIgG), IgM (IFIgM), C3 (IFC3) and C4 (IFC4). Those were correlated with analytical and clinical variables. Imaging results were subdivided into subclasses (turning the results into discrete variables): HC (mesangial or endocapillary) 0 represents absent HC, HC1 in ≤50% of glomeruli and HC2 in >50%; IFTA0 represents IFTA in ≤10% of glomeruli, IFTA1 in 11-25%, IFTA2 in 26-50% and IFTA3 >50%; in IF (IgA,G or M, C3 or C4)0 represents absent staining and 1-3 representing a gradual proportional intensity scale of IF staining (being 1 the lowest intensity and 3 the highest).

Serum creatinine was correlated with IFTA (+0.425mg/dL per IFTA level) as was eGFR (-38.9% eGFR per level). No correlation was found between laboratorial variables or remission status and HC (mesangial or endocapillary) or IF staining (IgA, G or M, C3 or C4) (Table II).

Therapeutical Decision and Clinical Outcomes

Median time to IST was 99 days (IQR 13-190). At the start of IST, patients displayed 1.01mg/dL median serum creatinine (IQR 0.78-1.48), 74mL/min/1.73m^2 median eGFR (47.9-105.3) and a 6.9g/g creatinine median proteinuria (4.9-10) (Table I).

After 2 years, 8 patients had NR (25%), 13 PR (41%) and 11 CR (34%). Remission status was not significantly different (p=0.560) between IST (6 CR, 10 PR and 6 NR) and non-IST (5 CR, 3 PR and 2 NR) patients. In the 10 non-IST patients, 5 of them presented SR (50%) (Table III).

Time to IST was not significantly different (p=0.451) between remission states: CR had a median interval of 13 days (IQR 11-134), PR 125 days (IQR 31-190) and NR 105 days (IQR 70-302). 11 patients were treated with a Ponticelli scheme (50%), 9 with cyclosporin-A (41%) and 2 with rituximab (9%). No rituximab patients displayed CR (1 NR and 1 PR). There was no significant difference between IST lines, regarding remission state after 2 years (p=1) (Table III).

Considering the log-transformed proteinuria trajectories (Figure 1 and 2), the CR group had the widest descent in annual proteinuria (- 1.79g/g creatinine/Year), followed by the PR (-0.69g/g creatinine/Year), and then NR (-0.18g/g creatinine/Year). NR and PR differed significantly (p=0.024), as did NR and CR (P<0.001).

Predictors of proteinuria

No significant correlation was found between baseline proteinuria and gender, age, baseline serum creatinine, IST or histological scores (Table IV). No correlation was found between the annual variation of proteinuria and age, gender, serum creatinine, initial presence/absence of NS, IST or histological scores (Table IV).

Age ≥60, female gender and baseline Serum Creatinine ≥1mg/dL were associated with a decreasing profile of proteinuria. Mesangial HC was associated with a significant increase in annual proteinuria. (Table IV).

Renal Survival

Patients were, once again, divided according to their 2-year remission status and. NR had a 5-year renal survival of 56%, PR 88% and CR 100%. NR was significantly more like to reach the composite outcome than the PR (p=0.010) and the CR (p=0.006) groups, but there was no statistically significant difference between the PR and the CR groups (p=0.274).

Two independent predictors for the composite outcome were identified: baseline serum creatinine ≥1.00mg/dL (13.755x higher risk (P=0.024)) and PR (0.040x lower risk (P=0.035), when compared to NR) (Table V).

DISCUSSION

This study allowed updated insight about the characteristics of MN patients in a contemporary cohort and highlights relevant clinical predictors of renal outcomes.

Median diagnostic age was 61.8 years, matching previous series (peak diagnostic age for MN is 45-80 years[10,11]). Our population was 41% female (a lower male-to-female dominance than the usual 2:1 ratio[14]). Females were linked with decreased annual proteinuria (-0.38g/g creatinine), matching previous findings that females with MN show improved remission and renal survival[83].

72% of patients present with NS, with 5.8g/g creatinine median proteinuria matching previous series (NS incidence of 60-80%)[3,4,7,21]. Baseline serum creatinine was 0.97 mg/dL in median, representing an 80.8mL/min/1.73m^2 median eGFR. Although the median patient presents with normal kidney function, 44% present initially reduced eGFR (<60mL/min/1.73m^2), superior to the 10-20% seen in previous series[4,7]. Our population presented more hypertension (63%) than other series (25-30%), which is associated with lower eGFR and worse renal outcomes[21]. 13% of our patients presented with diabetes mellitus, superior to European (8.0%) 2014 rates[84], increasing the number of reduced eGFR patients.

Surprisingly, higher baseline serum creatinine was correlated with decreased annual proteinuria. Elevated serum creatinine reflects decreased renal function, widely associated with MN progression. Proteinuria may be falsely decreased, hidden behind this decrease, creating a fake sign of improved outcome[85,86]. Like previous series, baseline creatinine ≥1mg/dL was determined to be an independent risk factor for our composite outcome.[87]

Our study had a reasonable surveillance time (median follow-up time of 3 years 6 months), in comparison with other retrospective cohorts studies, like Qin's[88] and Chavarot's[89] (2 years 6 months) Yamaguchi's[90] (3 years and 8 months) and Rozenberg's [91](4 years), although highly variable follow-ups have been used, from 7 years in Gupta's study[85] to only 15 months , in Wang's[92].

Controversy exists concerning therapy schemes, but IST initiation is recommended in elective cases by 6 months (180 days). Our median number of days until IST was a precocious 99. This may be justified by the 44% patients with decreased eGFR (attempting to avoid worse renal outcomes)[85]. IST population, at the start of IST, maintained a median proteinuria in the nephrotic range (6.9g/g creatinine), supporting this early therapy induction.

Although not significant, CR patients tend to initiate IST earlier (median 13 days) than PR (125 days) and NR patients (105 days). Yamaguchi's study displayed improved remission (compared to ours) and 21 days median interval to IST[90]. A Taiwanese study showed improved remission in the 1st year of follow-up in patients that start IST ≤6 months, rather than >6 months, which may justify this tendency (and motivate a revision of protocols).[93]

As expected, IST Individuals were more likely to present with NS than non-IST. Despite this, no statistically significant difference was found in baseline proteinuria or eGFR, between IST and non-IST individuals.

Higher baseline proteinuria has been correlated with worse remission states, lack of SR[2,94] and increased likelihood of IST[91]. Still, KDIGO guidelines only recommend IST in sustained nephrotic-range proteinuria after 6 months of SC[51] (as it predicts renal outcomes and CKD risk more clearly[95]). However emerging data point to the prognostic value of quantitatively measuring PLA2R antibody levels and in positive patients, high antibody levels appear to be correlated with no remission. In this cases IST may be started early.

IST patients seem to have a (nonsignificant) higher eGFR (89.3mL/min/1.73m^2) than non-IST (57.6). The 17.8 years of median age difference may contribute to this difference (eGFR declines 1mL/min/year[96]). Most likely, it reflects physicians' hesitation to expose patients with decreased eGFR's to IST, requiring dosing-adjustments or prohibiting their usage (e.g. serum creatinine >3.5mg/dL with alkylating agents[51]). Updates to KDIGO guidelines[59] recommend use of IST if recommended, independently from eGFR or serum creatinine, to stabilize renal function, increasing renal survival (preferably combining alkylating agents and corticosteroids[97]).

Histologic parameters confirmed their predictive relevance. The association between IFTA, increased serum creatinine and decreased eGFR has already been documented[98]. Any kidney-affecting disease can cause IFTA, making it an accurate representation of chronic kidney damage[99], that leads to worse MN outcomes[100] and loss of renal function[101]. Mesangial HC (a measure of glomerular sclerosis[102]), was correlated with an increase in annual proteinuria (+0.4 g/g creatinine/unit of HC), therefore, an association with worse renal outcomes. Previously, MN patients with these biopsy aspects showed lower survival rates at 24 months (and more arterial hypertension) [103]. Careful histological evaluation of these parameters is, therefore, clinically useful as a prognostic tool.

Remission (PR or CR) after 2 years was achieved in 75% of patients, with no significant difference between IST (72,3%) and non-IST (80%). Since randomized trials are scarce, results may depend on bias, on biopsy and therapy prescription. Yamaguchi's study[90] displayed remission rates of 87.7% after 10-years and Rozenberg's[91] of 81% after 4 years (but neither search for discrepancies between IST and non-IST). In the latter [91] all IST patients were administered alkylating agents, that show higher remission rates, contributing to the observed improved renal outcomes. Rozenberg also created a "high risk" patient group (proteinuria >6g/day, eGFR<60mL/min/1.73m^2 or severe disabling/life-threatening NS symptoms). Those in that category would immediately start IST, leading to a precocious ("time to IST" is unavailable in the study) and aggressive approach, potentially constituting a selection bias. Similarly, Yamaguchi's study[90] may display improved renal outcomes because of their reduced time to IST (21 days). There was no evaluation of remission

after 2-years, but after 1 year 38% had reached CR, a similar (but superior) value compared to our 34% CR after 2 years.

Gupta's study[85] compared remission (PR and CR) between IST (58%) and non-IST(46%). No significant variation was found between the 2. Only 1 patient (3%) in our database was non-white, comparing with 24% Black and 31% Asian in the English one. In Gupta's study (as in previous ones[104]), Black patients displayed worse renal outcomes, would more frequently progress to ESRD (adjusting for albuminuria, diabetes and hypertension, suggesting the presence of a genetic component), potentially contributing to Gupta's worse remission rates. Also, our patients were more frequently exposed to alkylating agents (50% vs 36% in the British cohort), improving the likelihood of remission.

No significant difference in remission was found between IST lines. The Ponticelli scheme had a 72,7% remission after 2 years, superior to previous series using (60-65% remission after 10 years[65,67]). Despite the difference in time frames, SR tends to occur in ≤14 months and the IST lasts for 6 months, so, from 2-10 years, remission states are less likely to improve significantly. 77,8% of patients treated with cyclosporine-A reached remission at 2-years of follow-up, an improvement, comparing with other series (around 70-75%[68,69]). RTX displayed a 50% remission rate, but only 2 patients were treated with RTX, rendering comparisons to other studies pointless.

PR or CR showed better renal survival than NR; PR was found to be an independent factor for better renal survival (PR, were 0.04x less likely than NR to reach the composite outcome). The lack of association between CR and renal survival is justified by the absence of the composite outcome in CR patients (representing the positive impact of CR in renal survival[105]).

PLA_2R1-antibody was found in 4 out of 6 tested patients' serum samples. In our study, the number of tested patients is still limited and desirably more data will be gathered to increase our knowledge of its diagnostic accuracy. Given the variable presentation (some PLA_2R1-related PMN may be initially serum-negative)[46] it might also be more useful during follow-up (in patient outcomes management).

Our study is inherently limited to a small patient sample, but as data collection was done from an university hospital database, we benefited from a detailed registry of the studied variables (including rigorous histological scoring) and this study can help promoting a more accurate MN clinical investigation.

The inclusion of the new biomarker phospholipase A2 receptor (PLA2R1) antigen in the predictive model will hopefully increase the ability to individualize therapy and better predict the prognosis.

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TABLES

Table I – Correlation of clinical and laboratorial values with decision to initiate (or not) IST

	Baseline				At start of IST
	Total N=32	No IST N=10 (31%)	IST N=22 (69%)	P	IS N=22
Age, median (IQR)	61.8 (44.2-68.7)	68.4 (63.6-73.4)	50.6 (43.0-64.6)	**0.017**	
Female, n (%)	13 (41)	5 (50)	8 (36)	0.467	
Year of biopsy, median (IQR)	2012 (2010-2016)	2012 (2010-2013)	2014 (2009-2016)	0.347	
Weight, median (IQR)	72.5 (63.5-84.3)	69.5 (63.5-77.3)	78.3 (63.5-85)	0.542	
Days until IST treatment, median (IQR)					99 (13-190)
Serum creatinine, median (IQR)	0.97 (0.74-1.41)	1.13 (0.84-2.01)	0.87 (0.7-1.36)	0.155	1.01 (0.78-1.48)
eGFR, median (IQR)	80.8 (44.3-109.0)	57.6 (32.2-85.9)	89.3 (51.1-111.8)	0.113	74.0 (47.9-105.3)
Proteinuria, median (IQR)	5.8 (4.3-8.1)	5.8 (4.1-6.7)	5.9 (4.5-9.7)	0.515	6.9 (4.9-10.0)
Serum albumin, median (IQR)	2.52 (2.13-3.1)	2.95 (1.89-3.46)	2.47 (2.18-2.75)	0.569	
Nephrotic syndrome, n (%)	23 (72)	4 (40)	19 (86)	**0.013**	
Hypertension, n (%)	20 (63)	8 (80)	12 (55)	0.248	
Number of antihypertensive drugs, median (IQR)	1 (0-2)	3 (0-3)	1 (0-2)	0.167	
ACEi/ARB use, n (%)	14 (44)	6 (60)	8 (36)	0.267	
Diabetes, n (%)	4 (13)	2 (20)	2 (9)	0.572	
Dyslipidemia, n (%)	21 (66)	6 (60)	15 (68)	0.703	
Follow-up (years), median (IQR)	3.5 (1.9-6.3)	2.7 (0.8-6.1)	3.6 (2.4-6.5)	0.309	

ACEi – Angiotensin Convertase-enzyme inhibitor; ARB – Angiotensin II Receptor Blocker; eGFR – estimated glomerular filtration rate; IST- immunosuppressive treatment

Table II – Correlation between histological and laboratorial variables and remission status

	Total N=32	Spearman correlations Rho				
		Serum creatinine	eGFR	Proteinuria	Serum albumin	Remission status (0-2)
Number of glomeruli, median (IQR)	12 (7-18)	-	-	-	-	-
HC mesangial, n (%) 0 1 2	 23 (72) 7 (22) 2 (6)	-0.263	0.299	-0.187	-0.065	-0.097
HC capilar, n (%) 0 1	 31 (97) 1 (3)	0.010	-0.010	-0.165	0.185	-0.031
IFTA, n (%) 0 1 2 3	 19 (59) 8 (25) 4 (13) 1 (3)	**0.425**	**-0.398**	0.220	0.124	-0.311
IF_IgA, n (%) 0 1 2	 20 (63) 10 (31) 2 (6)	0.080	-0.106	0.284	-0.032	0.180
IF_IgG, n (%) 0 1 2 3	 4 (13) 1 (3) 7 (22) 20 (63)	-0.109	0.164	-0.211	-0.057	0.208
IF_IgM, n (%) 0 1	 22 (69) 10 (31)	-0.164	0.197	-0.073	0.037	0.094
IF_C3, n (%) 0 1 2 3	 6 (19) 9 (28) 13 (41) 4 (13)	0.119	-0.059	-0.124	-0.113	0.173
IF_C4, n (%) 0 1 2	 26 (81) 4 (13) 2 (6)	-0.227	0.246	-0.180	0.274	0.054

eGFR – estimated glomerular filtration rate; HC – hypercellularity; IFTA – Interstitial fibrosis and tubular atrophy; IF – immunofluorescence; Ig – immunoglobulin

Table III – Correlation between treatment and remission status

	Remission status at 2-years				
	Total	No remission	Partial remission	Complete remission	
Total, n (%)	-	8 (25)	13 (41)	11 (34)	-
No IST, n (%)	-	2 (25)	3 (23)	5 (45)	0.560
IST, n (%)	-	6 (75)	10 (77)	6 (55)	
Ponticelli, n (%)	11 (50)	3 (50)	5 (50)	3 (50)	1
CsA, n (%)	9 (41)	2 (33)	4 (40)	3 (50)	1
Rituximab, n (%)	2 (9)	1 (17)	1 (10)	0	1
Days to IST, median (IQR)	-	105 (70-302)	125 (31-190)	13 (11-134)	0.451

CsA – Cyclosporin-A; IST – immunosuppressive therapy;

Table IV – Correlation between annual proteinuria and clinical, laboratorial and histological variables (univariate and multivariable linear mixed regression model)

	Univariate			**Multivariable***		
	Unstandardized β	**95% CI**	**P**	**Unstandardized β**	**95% CI**	**P**
Time (yearly)	-0.97	-1.18-(-0.75)	**<0.001**	-0.87	-1.20-(-0.54)	**<0.001**
Age≥60 (vs <60)	0.60	-0.01-1.22	**0.055**	0.35	-0.27-0.96	0.267
Time*Age≥60 (vs <60) ***Time*Age<60*** ***Time*Age≥60***	-0.34 -0.78 -1.13	-0.76-0.07 -1.09-(-0.48) -1.42-(-0.84)	**0.107**	-0.61 -0.66 -1.26	-1.04-(-0.17) -0.95-(-0.37) -1.55-(-0.98)	**0.006**
Female (vs male) gender	-0.17	-0.82-0.48	0.609	-	-	-
Time*Female (vs male) gender ***Time*Male*** ***Time*Female***	-0.41 *-0.81* *-1.22*	-0.84-0.01 *-1.07-(-0.54)* *-1.55-(-0.89)*	**0.057**	-0.38 -0.82 -1.20	-0.75-(-0.01) -1.06-(-0.58) -1.50-(-0.91)	**0.044**
SCr at bx≥1.00 (vs <1.00)	0.48	-0.09-1.04	**0.097**	0.28	-0.32-0.88	0.364
Time*SCr at bx≥1.00 (vs <1.00) ***Time*SCr≥1.00*** ***Time*SCr<1.00***	0.32 *-1.10* *-0.78*	-0.10-0.74 *-1.38-(-0.82)* *-1.10-(-0.46)*	**0.134**	0.76 -1.30 -0.54	0.32-1.20 -1.56-(-1.03) -0.85-(-0.23)	**0.001**
Nephrotic syndrome (vs no)	0.37	-0.23-0.98	0.225	-	-	-
Time*Nephrotic syndrome (vs no) ***Time*Non-nephrotic*** ***Time*Nephrotic***	0.19 *-1.11* *-0.92*	-0.28-0.66 *-1.50-(-0.71)* *-1.16-(-0.67)*	0.432	-	-	-
IS (vs no IS)	0.38	-0.25-1.00	0.241	-	-	-
Time*IS (vs no IST) ***Time*No IST*** ***Time*IST***	0.15 *-1.08* *-0.93*	-0.34-0.64 *-1.51-(-0.66)* *-1.18-(-0.69)*	0.545	-	-	-
HC Mesangial per unit	-0.19	-0.60-0.22	0.360	-	-	-
Time*HC Mesangial per unit	0.50	0.17-0.84	**0.003**	0.40	0.14-0.66	**0.002**
IFTA per unit	0.25	-0.17-0.67	0.250	-	-	-
Time*IFTA per unit	0.10	-0.19-0.39	0.491	-	-	-
IF_IgG per unit	-0.12	-0.41-0.16	0.397	-	-	-
Time*IF_IgG per	-0.13	-0.34-0.08	0.219	-	-	-

unit						
IF_IgA per unit	0.19	-0.31-0.68	0.463	-	-	-
Time*IF_IgA per unit	-0.44	-0.77-(-0.12)	**0.007**	-0.21	-0.50-0.08	0.149
IF_IgM per unit	-0.01	-0.68-0.66	0.976	-	-	-
Time*IF_IgM per unit	-0.29	-0.74-0.16	0.204	-	-	-
IF_C3 per unit	-0.06	-0.40-0.28	0.736	-	-	-
Time*IF_C3 per unit	-0.15	-0.38-0.08	0.173	-	-	-
IF_C4 per unit	-0.24	-0.85-0.36	0.426	-	-	-
Time*IF_C4 per unit	0.07	-0.28-0.42	0.707	-	-	-
Constant				1.20	0.77-1.62	<0.001

HC – Hypercellularity; IF – immunofluorescense; Ig – Immunoglobulin; IFTA – interstitial fibrosis and tubular atrophy; IST – immunossupressive treatment; SCr: serum creatinine

Table V – Independent risk factors for the composite outcome and their hazards ratio (using Cox proportional hazards regression)

	HR	95% CI	P
SCr at baseline ≥1.00 (vs <1.00)	13.755	1.419-133.334	0.024

	HR	95% CI	P
Remission status			0.024
No remission	Reference		
Partial remission	0.040	0.002-0.795	0.035
Complete remission	*No events*	-	-

SCr = serum creatinine.

GRAPHICS

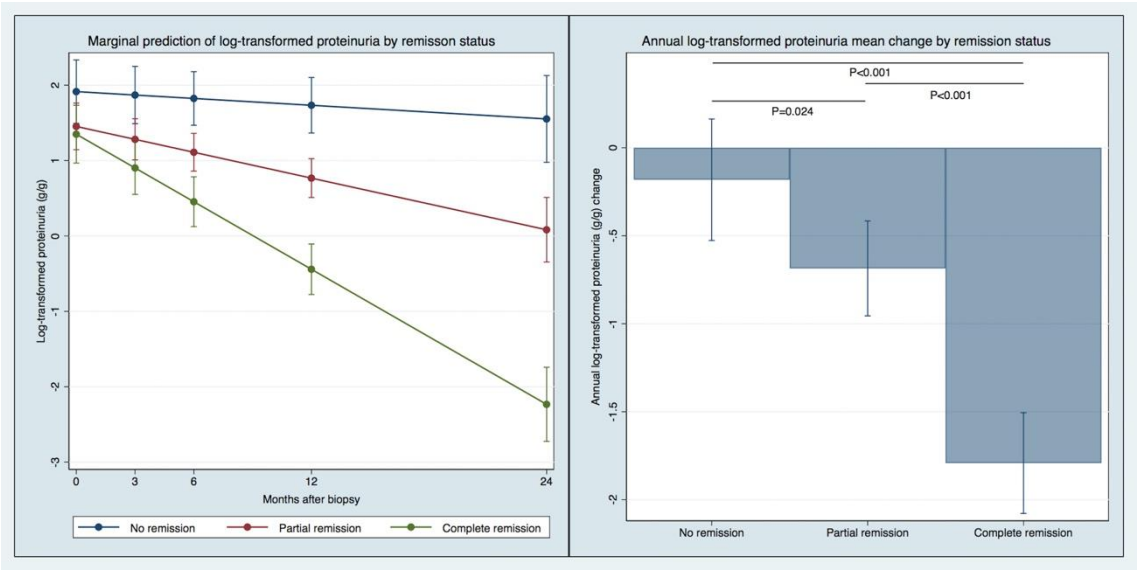

Figure 1 and 2- Correlation between annual variation of (log) proteinuria and remission status

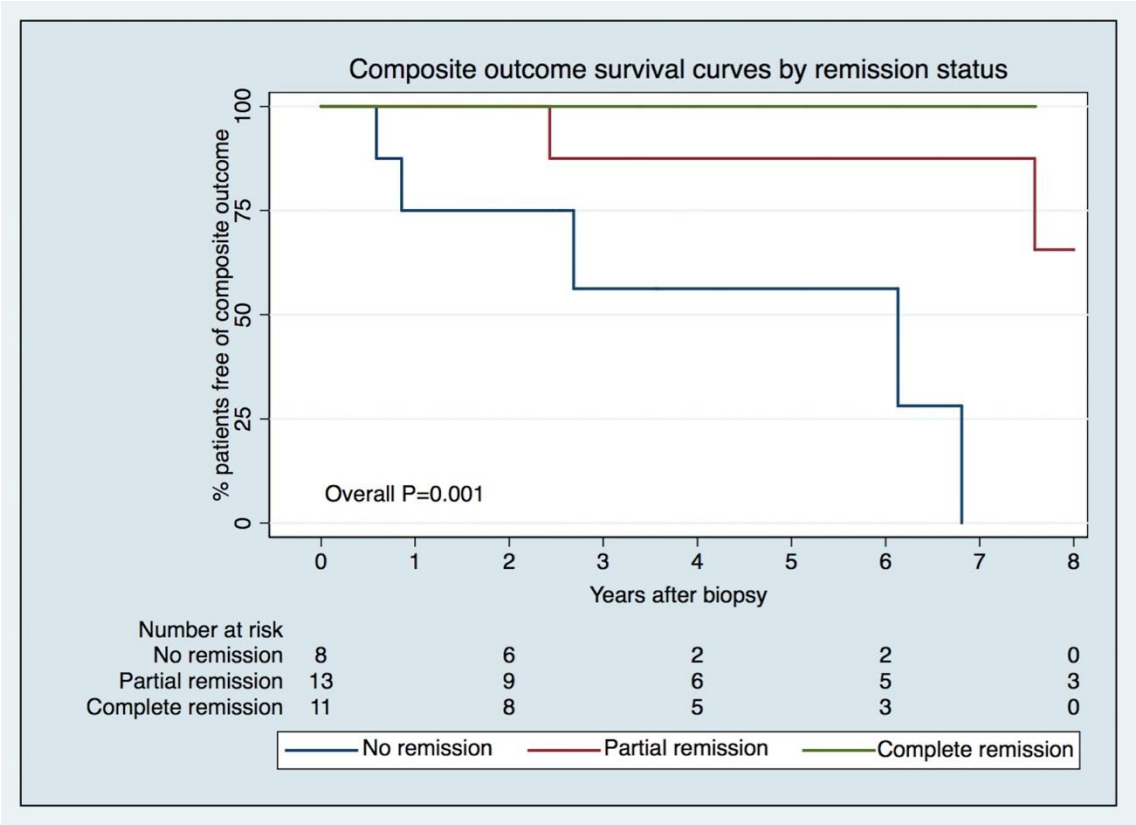

Figure 3 - Renal survival analysis by Kaplan-Meier.

POSFÁCIO

O abstract deste trabalho foi aceite para apresentação no Encontro Renal de 2020.

De: **Abstracts** <abstracts@bbg.pt>
Date: sexta, 1/05/2020, 17:06
Subject: Predictors of remission status in a Membranous Nephropathy cohort
To: <sofia.fersantos@gmail.com>

Exma (o),

O respetivo Abstract foi selecionado para apresentação ao Encontro Renal de 2020 (que decorrerá no Centro de Congressos do Marinotel - Vilamoura de 29 a 31 de outubro de 2020).
No início de setembro será informado sobre a data e tipo de apresentação (poster ou comunicação oral) deste mesmo abstract.

A Sociedade Portuguesa de Nefrologia agradece o seu interesse e a sua participação no Encontro Renal 2020,

Aguardando vê-lo(a) em Vilamoura,

Os melhores cumprimentos,
Secretariado
Encontro Renal 2020

--
MCPTS,

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