UNIVERSITY OF MEDICINE AND PHARMACY
"CAROL DAVILA", BUCHAREST
DOCTORAL SCHOOL
MEDICINE

EPIDEMIOLOGY AND PROGNOSIS OF BIOPSY-PROVEN GLOMERULOPATHIES IN ADULTS

PhD THESIS SUMMARY

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2023

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RESEARCH HYPOTHESES AND OBJECTIVES

The extended spectrum of diseases of the glomerulus that have heterogeneous etiology, pathogenesis, clinical manifestation, and treatment is grouped under the terminology of "glomerulopathies". These pathological conditions of the glomerulus have a low global prevalence in the general population, with important geographic variability, being considered rare diseases even the most common glomerulopathies [1], [2].

However, non-diabetic glomerulopathies (GP) account for approximately 15% of cases of Chronic Kidney Disease (CKD) – an important cause of morbidity and mortality worldwide, with increasing incidence and notable socio-economic impact [3]. Moreover, glomerulopathies are the third leading cause of renal replacement therapy initiation (RRT) in developed countries, after diabetes mellitus and hypertension [3], [4]. In our country, in 2019, GP represented 17% of end stage kidney disease [5]. In developing countries from Asia or Africa, GP is the first cause of RRT [6].

Currently, epidemiological data on the frequencies of GP in South-Eastern Europe are limited, and those in our country come from northern and western regions of Romania, which serve only 23% of the country's adult population [7].

The clinical presentation is not specific for the diagnosis of GP, as there may be overlaps between the types of glomerular and tubulo-interstitial or vascular clinical manifestations, the differentiation of acute from chronic can sometimes be difficult to establish, and the severity of symptoms does not always correspond to the severity of histological lesions [8]. Therefore, the form of clinical presentation is often mandatory to be completed by the histologic examination. However, it should be taken into account that each histological pattern can be common to several types of glomerulopathies. Therefore, clinical-nephropathology integration is essential for a correct and complete diagnosis [8].

The clinical-nephropathology integration also allows for the evaluation of the local medical practice and, through comparisons with other centers with greater experience, can contribute to the improvement of medical services for the benefit of the patient. Moreover, data on the epidemiology and anatomic-clinical characteristics of GP can be useful in developing strategies for early detection of the population at risk, as well as to increase the reference of patients to the nephrologist.

Along with the aging, there are physiological changes of the kidneys, including the glomerulus (glomerulosclerosis, mesangial expansion, thickening of the glomerular basement membrane) that lead to the physiological decrease of approximately 1 mL/min/year of the glomerular filtration rate after the age of 30 [9]. However, with the increase in life expectancy, the incidence of chronic kidney disease, including glomerulopathies, also increases among the elderly. However, data on the epidemiology and prognosis of glomerulopathies in this population are limited. Furthermore, it is not clear at this time whether age and possibly age-related glomerular changes negatively impact kidney survival in these patients.

On the other hand, diabetes mellitus (DM) is one of the most important current public health problems, with an increasing prevalence. DM is the first cause of initiation of renal replacement therapy and in the top ten causes of death [4], [10]. Thus, analyzing the types of glomerular lesions in diabetic subjects, identifying the clinical and prognostic predictors of non-diabetic lesions, as well as evaluating the role of diabetes in the progression of patients with non-diabetic glomerular diseases may contribute to improving the care of patients with DM.

Therefore, the primary objectives of the current thesis were:

1. Evaluation of the epidemiology of biopsy-proven glomerulopathies in south-eastern Romania;
2. Evaluation of the prognostic role of age on kidney survival in patients with biopsy-proven glomerulopathies;
3. Evaluation of the prognostic role of diabetes mellitus on kidney survival in patients with biopsy-proven glomerulopathies;
4. Evaluation of the prognostic role of diabetes mellitus on the overall survival in patients with biopsy-proven glomerulopathies.

RESEARCH METHODOLOGY

This was an observational, longitudinal, retrospective, single-center study conducted between January 1st 2008, and May 31st 2018.

Between Jan. 1st 2008 - Dec. 31st 2017, 1697 histopathological results from kidney biopsies (KB) in adults admitted to the Clinical Hospital of Nephrology "Dr. Carol Davila" were available.

The biopsy was performed under real-time ultrasound guidance, using an automated biopsy gun with a 16-gauge needle. The same pathologist analyzed all biopsies in light microscopy, immunofluorescence, and electron microscopy.

Between 1st May 2018 – 31st May 2018, all selected subjects were searched in the database of the Romanian Renal Registry (based on the personal identification code) to record the time of initiation of dialysis or renal transplantation. In addition, to determine whether patients had died, based on the personal identification code, they were searched in the Health Insurance Informatics Platform (http://siui.casan.ro:82/Asigurati/). For patients who died, the date of death was obtained either from the hospital's computer system (for patients who died in the hospital), or by phone contact of the relatives or by approximating the date of death according to the date of the last evaluation in the Clinic.

Inclusion criteria were:

1. age >18 years;
2. histologic diagnosis of glomerulopathy after percutaneous kidney biopsy.

Exclusion criteria were:

1. repeated kidney biopsy during the study period;
2. renal graft biopsy;
3. other type of tissue than kidney;
4. absence of glomeruli for examination in all the three methods (light microscopy, imunofluorescence and electron microscopy);
5. absence of laboratory data;
6. dialysis initiation for more than 1 month prior to kidney biopsy.

Using the selection criteria, the final analysis included 1200 subjects (**Figure 1**).

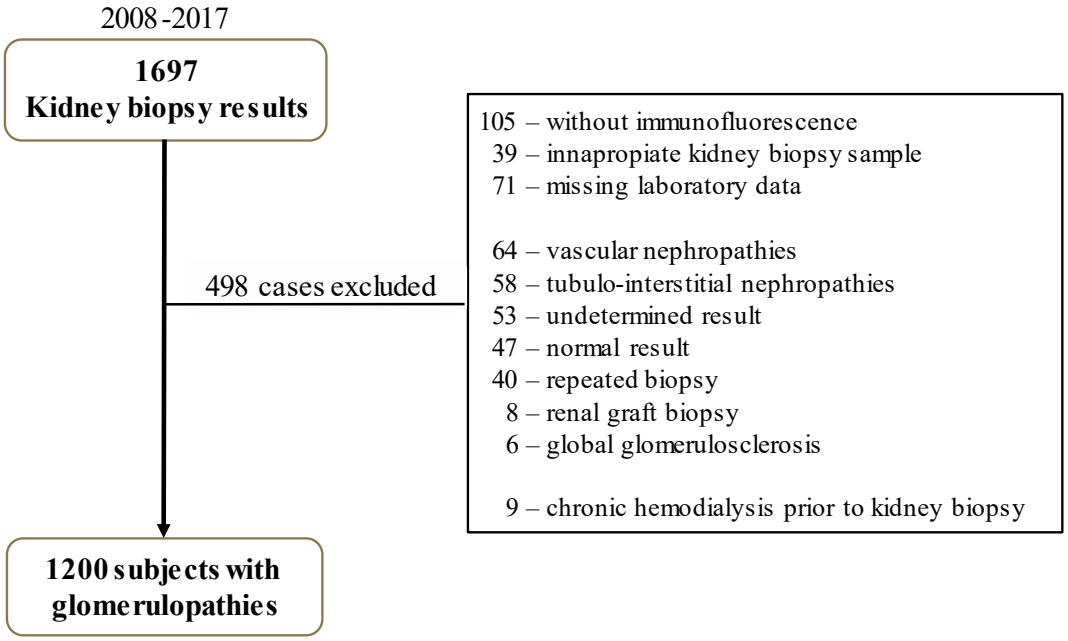


Figure.1. Study flowchart

End-points of the study were:

1. Kidney survival: need of renal replacement therapy – hemodialysis, peritoneal dialysis or kidney transplant.
2. Patients' survival – all cause mortality.

Statistical analyses were performed with IBM SPSS software version 20, Analyse-it Medical Edition, and SAS Studio.

For the descriptive analysis, results were reported as mean and 95% confidence interval of the mean or median and 95% confidence interval, depending on distribution (parametric or non-parametric). The Shapiro-Wilk test was used to assess whether continuous variables had parametric or non-parametric distribution.

For the comparisons, student T-test was used for groups with continuous parametric distribution, the Kruskall-Wallis test was used for comparing two or more groups with non-parametric distribution and the Chi-square test for qualitative variables.

The annual incidence was expressed as the total number of annual cases reported to the population at risk and expressed per million population/year (pmp/year). According to the data of the census carried out in Romania in 2011 [11], a number of 7261401 adults were considered

as population at risk, i.e. the total population of the counties in the south and south-east of Romania.

The Kaplan-Meier method was used to evaluate the time to one of the events of interest (RRT initiation or death), and the log-rank test was used for the comparisons. The multivariate Cox proportional hazard analysis was used to evaluate the independent risk factors for RRT initiation or death.

A competing risk factor is that event (in the present studies - death) that can prevent the occurrence of the final event of interest (RRT). In the competing risk analysis, subjects experiencing the competing event have zero risk of experiencing the event of interest, compared to Kaplan-Meier analysis where the probability of the event of interest is similar regardless of the presence of other independent prior events, leading to overestimation of the cumulative incidence of the event of interest [12]. To identify independent predictors of the event of interest in the presence of the competitive event, the subdistribution hazard function obtained from the Fine-Gray model [13].

For all the tests, a p value < 0.05 was considered statistically significant.

The study was conducted in accordance with the Declaration of Helsinki and the protocol was approved by the local Ethics Committee. The need for informed consent was waived due to the retrospective nature of the study.

STUDY 1 – EPIDEMIOLOGY OF GLOMERULOPATHIES IN ADULTS FROM SOUTH-EAST ROMANIA

This study had three parts. In the first part we analyzed the characteristics of subjects with kidney-biopsy proved glomerulopathies from south and south-east Romania. In the second part the dynamic of evolution of glomerulopathies during a decade (2008-2017) was evaluated. In the third part the frequency and characteristics of glomerulopathies from south and south-east Romania and Norway were comparatively analyzed.

The annual kidney biopsy rate was 25.9 pmp/year for the 2008-2017 interval which places us among in the last places in Europe regarding the kidney biopsy pracice.

The analyzed group (N=1200) was represented by middle-aged patients, most of whom were in their 5th decade of life at the time of KB (79% between 31-64 years, 8% >65 years), with a slight male predominance.

Nephrotic syndrome was the main indication for KB in the study cohort, recorded in more than a third of subjects, followed by chronic nephritic syndrome (in a quarter of patients), acute nephritic syndrome and chronic renal failure of unspecified cause (approximately 1 in 10 patients each).

Primitive glomerulopathies represented by IgA nephropathy (IgAN), membranous nephropathy (MN), minimal change disease (MCD) and focal and segmental glomerulosclerosis (FSGS) accounted for half (50.9%) of all diagnosed glomerulopathies, the rest being secondary GP (43.9%), respectively hereditary GP (5.2%). The first cause of secondary GP was diabetic nephropathy. Men were most frequently diagnosed with primary nephropathies - IgAN, followed by MN and MCD, while the first histological diagnosis in women was lupus nephritis, followed by IgAN and MCD.

To analyze the dynamics of GP frequency during the decade, the subjects were divided into 3 groups according to time periods: period 1 (2008-2010; n=157), period 2 (2011-2014; n=452) and period 3 (2015-2017; n=591). During the three investigated periods an important increase in the frequency of diagnosis of GP was observed: between 205-2017 four times more GP were diagnosed compared to the first period.

Subjects diagnosed with GP in the first analyzed period were significantly younger compared to those biopsied in the last 2 periods. In all three periods, around 70% of the subjects

were between 31-64 years old, but in the period 2008-2010 a significantly higher proportion of 18-30 year olds (19.7%) is noted compared to the other two periods (11.5% in 2011-2014 vs. 9.6% in 2015-2017; $p<0.001$) and a lower frequency of the elderly (>65 years; 8.3% vs. 17.7% in 2011-2014 vs 17.9% in 2015-2017). This suggests the extension of the indication for KB also in the elderly with increasing experience.

The age difference was also reflected by the higher comorbidity index in the last two periods compared to the first period, being identified with a higher frequency of arterial hypertension, diabetes mellitus. and manifestations of systemic atherosclerosis. The subjects analyzed from the last period had significantly lower renal function compared to those from the first period.

Nephrotic syndrome was half of the KB indications for the subjects in the first period and just under half for the second period, while in the latter period it was the indication for PBR for only one third of the subjects. In period 3 there was a higher proportion of subjects who were biopsied for CKD of unknown reason.

Thus, it was evident a tendency in the change of the KB indications, which also led to the sustained increase in the biopsy rate during the 3 analyzed periods. Consecutively, the stable increase in the incidence of all histological substrates of GP during the last 2 periods compared to the first period is observed. Along with the increase in the biopsy rate, a tendency to change the spectrum of glomerulopathies diagnosed in the 3 periods was recorded with an increase in the frequency of secondary glomerulopathies detected in the last period compared to the first period ($p=0.03$).

No difference was observed regarding the frequency of the types of primitive glomerulopathies, regardless of the period analyzed, IgA nephropathy being the main histological substrate, followed by MCD in the period 2008-2010 and membranous nephropathy in the periods 2011-2014 and 2015-2017. Hereditary GP were also diagnosed in a similar proportion ($p=0.8$).

Regarding secondary GP notable differences were found between the 3 analyzed periods. The most important change is the higher frequency of diabetic nephropathy, which tripled in the last period compared to the first analyzed period, becoming the first type of secondary GP diagnosed between 2015-2017 compared to the fourth type of GP diagnosed in 2008-2010.

From the comparative analysis of the data from our center with those from the Norwegian Renal Biopsy Registry, it appears that the biopsy rate in Norway was 6.5 times higher (130.5 pmp/year) compared to our center for the analyzed period (2008 -2014).

From the comparison of demographic data, it was noted that the subjects diagnosed with GP in Norway were older compared to those in Romania, a quarter of the selected patients being over 65 years old (26.6% vs. 15.3%; $p<0.001$). Young adults were in equal proportions in both analyzed groups (15%). A slight predominance of male patients is observed in both countries. Global kidney function was significantly lower among subjects with glomerulopathies in Norway, with a slightly higher proportion of advanced G4-5 categories observed (32% vs. 26% in Romania).

The main indication for KB in Norway was chronic nephritic syndrome, followed by nephritic-nephrotic syndrome and, only in third place, nephrotic syndrome. By comparison, in Romania the main indication of PBR was nephrotic syndrome in almost half of the subjects, followed by chronic nephritic and acute nephritic syndrome. The higher frequency of biopsy for asymptomatic urinary abnormalities and acute kidney injury in Norway are also notable.

In the analyzed period, the main GPs diagnosed in Romania were primitive glomerulopathies, first being IgA nephropathy, followed by membranous nephropathy and MCD, totaling 46% of all GPs. Similarly, IgA nephropathy was the first type of glomerulopathy diagnosed in a quarter of Norwegians, but was followed by secondary glomerulopathies, namely crescentic GN (15%) and diabetic nephropathy (12%). Among the crescentic GN detected in Norway, ANCA vasculitis prevail (82%), followed by anti-glomerular basement membrane GN in 8.6% of cases. Moreover, in Norway, a higher proportion of secondary GPs (50.2% vs. 43%, $p=0.004$).

STUDY 2 – OUTCOMES OF GLOMERULOPATHIES IN ELDERLY

In the second study, the utility of kidney biopsy and the kidney outcome of biopsy-proven glomerulopathies were assessed, targeting those aged 65 years and older.

We mention that the selection period of the subjects of the current study was between January 1st, 2010, and December 31st, 2016, since for this interval the most complete data on the changes in the treatment of patients were available.

Using the selection criteria, 875 patients were finally analyzed and divided into two groups: adults (18-65 years) and elderly (≥65 years) (**Figure 2**).

The patients were followed up from kidney biopsy until May 31st, 2018 to one of the two outcomes: end-stage kidney disease, death. The mean follow-up period was 71.1 (95% CI 68.2–73.9) months.

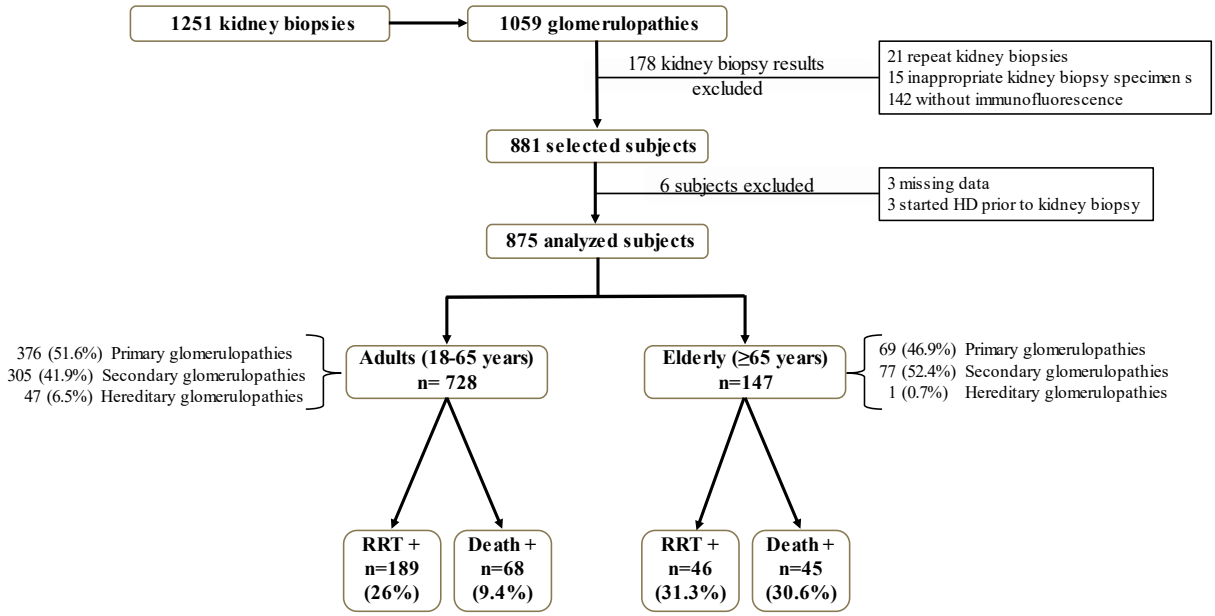


Figure 2. Study flowchart.
HD: hemodialysis; KB: kidney biopsy; GP: glomerulopathies; GS: glomerulosclerosis; RRT: renal replacement therapy

Most of the subjects in the elderly group were aged 65–74 years (79%), while one fifth (21%) were aged ≥75 years.

The referral for KB was different according to age groups: nephrotic syndrome was the main indication for biopsy in more than half of the elderly, but in only a third of adults, while chronic nephritic syndrome, acute kidney injury and CKD of unknown cause, were more commonly seen in subjects <65 years, suggesting different etiologies.

Biopsy-related complications, none of them severe, had a similar low incidence irrespective of age.

Secondary glomerulopathies were more frequent in the elderly (52.4% vs. 41.9%, p=0.004), amyloidosis (16%), followed by diabetic glomerulosclerosis (12%) being the most common. Membranous nephropathy accounted for one quarter of cases and was the most prevalent primary glomerulopathy in those aged >65 years, followed by MCD.

Elderly had significantly lower eGFR. Moreover, higher proteinuria and lower serum albumin were encountered in these subjects, reflecting the greater proportion of nephrotic syndrome. Similar percentages of hematuria and dysmorphic red blood cells were found, even though chronic nephritic syndrome was less common in elderly.

Angiotensin converting enzyme inhibitors and/or angiotensin receptor blockers were less often indicated (in less than one third) in elderly, while immunosuppressive medication was slightly more often prescribed in elderly, both before and after KB. The kidney biopsy result changed the therapy in almost half of the subjects, in similar proportions in both groups.

As expected, the mortality rate was higher in the elderly (OR 4.2; 95%CI 2.7 to 6.7; p<0.0001). In univariate time-dependent analysis (**Figure 3a**), patients aged ≥65 years had a lower survival than younger subjects [62.6 (95%CI 54.1 to 71.06) vs. 88.3 (95%CI 86.1 to 90.4) months, log rank p=0.001]. Secondary glomerulopathies, along with older age, higher comorbidity score and absence of RASi were the independent predictors of all cause-mortality.

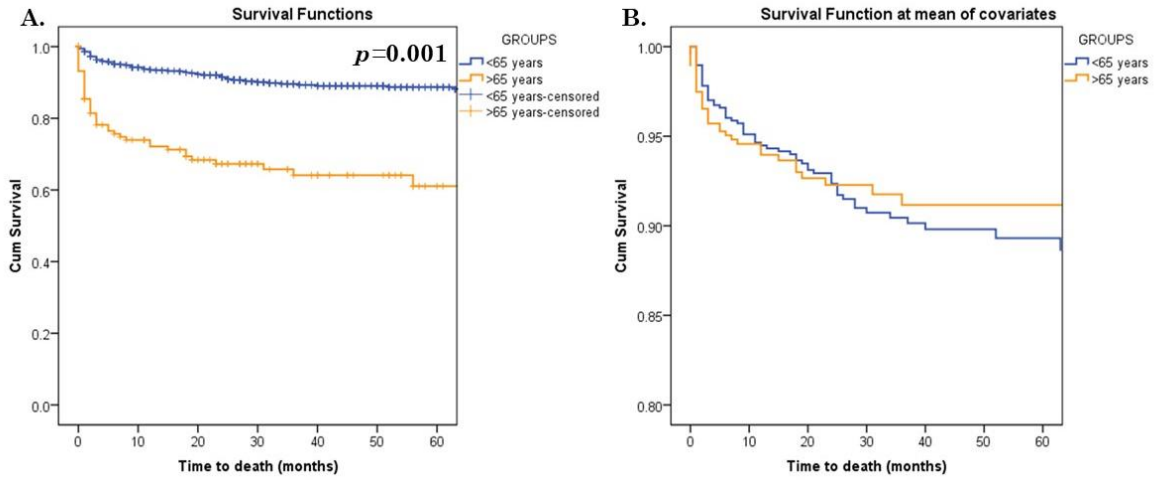
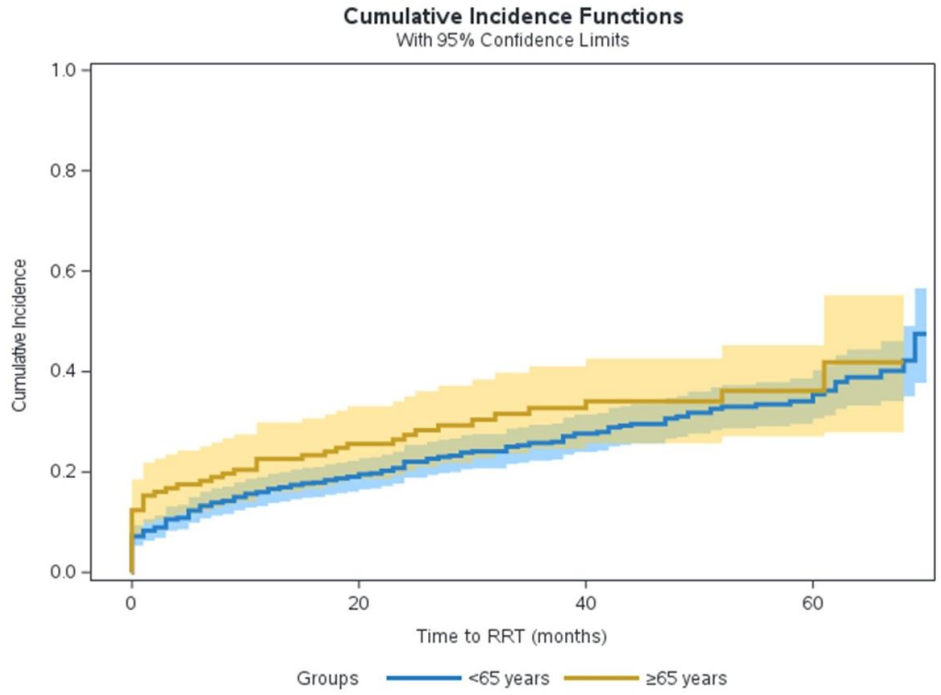


Figure 3. Unadjusted (a) and adjusted (b) patients' survival according to age: <65 years (blue line) versus ≥65 years (orange line);

RRT was started in similar proportions in both groups (OR 1.2; 95%CI 0.8 to 1.9; p=0.1).



Summary of Failure Outcomes				
Groups	**Failed Events (RRT+)**	**Competing Events (Death)**	**Censored**	**Total**
<65 years	189	68	471	728
≥65 years	46	45	56	147
Total	235	113	527	875

Figure 0. Cumulative incidence function (95% CI)) of kidney outcome <65 years (blue line) versus ≥65 years (orange line). RRT: renal replacement therapy

In the competitive risk time to event analysis where death was considered the competing event, elderly and those aged <65 years had similar kidney survival as [CIF 0.4 (95%CI 0.26-0.53) vs. 0.34 (95%CI 0.28 to 0.39), p=0.08] **(Figure 4)**. After adjusting for the risk factors for CKD progression, younger age seems to be associated with an increased rate of RRT (HR=0.98, p=0.03) in a subdistribution hazard model, along with hypertension, lower hemoglobin, lower eGFR and higher proteinuria**.**

STUDY 3 - NON-DIABETIC GLOMERULAR LESIONS IN DIABETIC KIDNEY DISEASE: CLINICAL PREDICTORS AND OUTCOME

In the third study we evaluated the frequency of non-diabetic glomerular lesions, clinical predictors and kidney outcome in a cohort of adults with Diabetic kidney disease.

The selection criteria were those previously presented in the Research Methodology section, to which we added:

Inclusion criteria: diabetes mellitus diagnosis.

Exclusion criteria:

- Steroid induced diabetes mellitus;
- Mixed histologic lesions (non-diabetic kidney lesions superimposed on diabetic nephropathy).

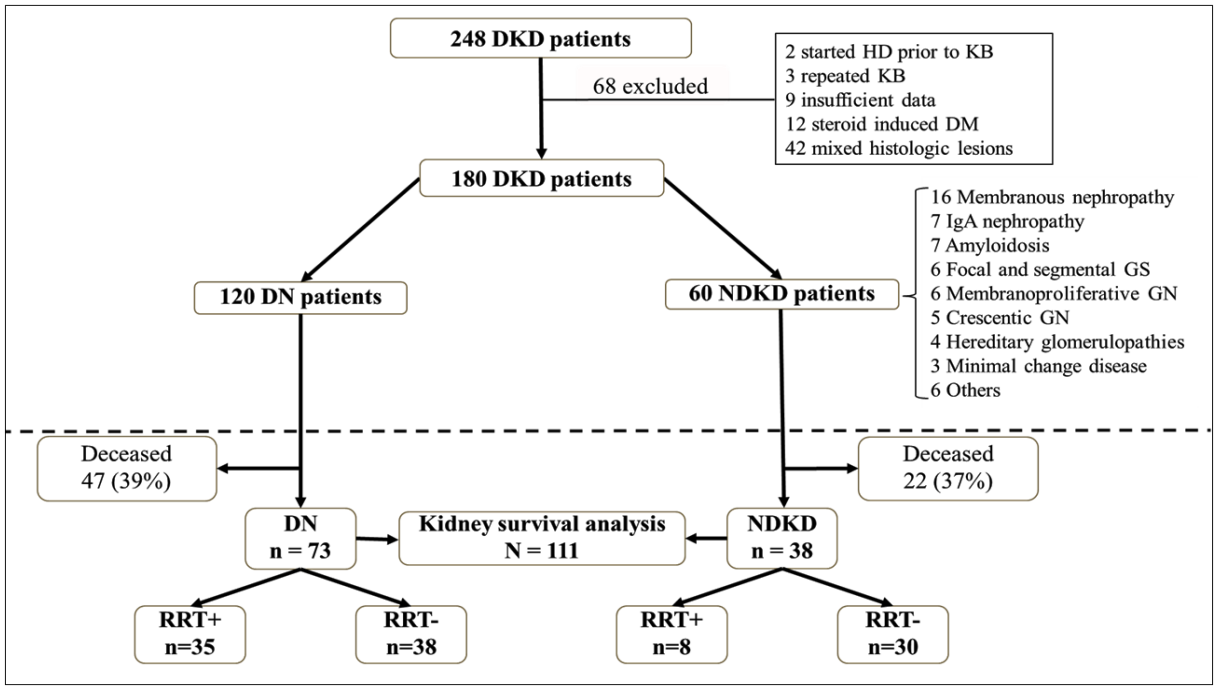


Figure 0. Study Flowchart
DKD: diabetic kidney disease; DM: diabetes mellitus; DN: diabetic nephropathy; GN: glomerulonephritis; GS: glomerulosclerosis; HD: hemodialysis; NDKD: non-diabetic kidney disease; RRT: renal replacement therapy; KB: kidney biopsy

Finally, 180 subjects were included (**Figure 5**).

Nondiabetic kidney disease (NDKD) was diagnosed in one third of the subjects. The most common glomerular pattern was membranous nephropathy (27%), followed by IgA nephropathy and amyloidosis (12% each).

The indication of biopsy differed according to study group: in the NDKD group, biopsy was indicated more frequently for nephrotic syndrome as in the DN group, while chronic nephritic syndrome, isolated proteinuria, and CKD of unknown cause, were more frequent indications in the DN group, suggesting distinct clinical presentation in NDKD and DN.

In the multivariate logistic regression analysis, shorter DM, absence of diabetic retinopathy and presentation as nephrotic syndrome were the independent predictors of NDKD.

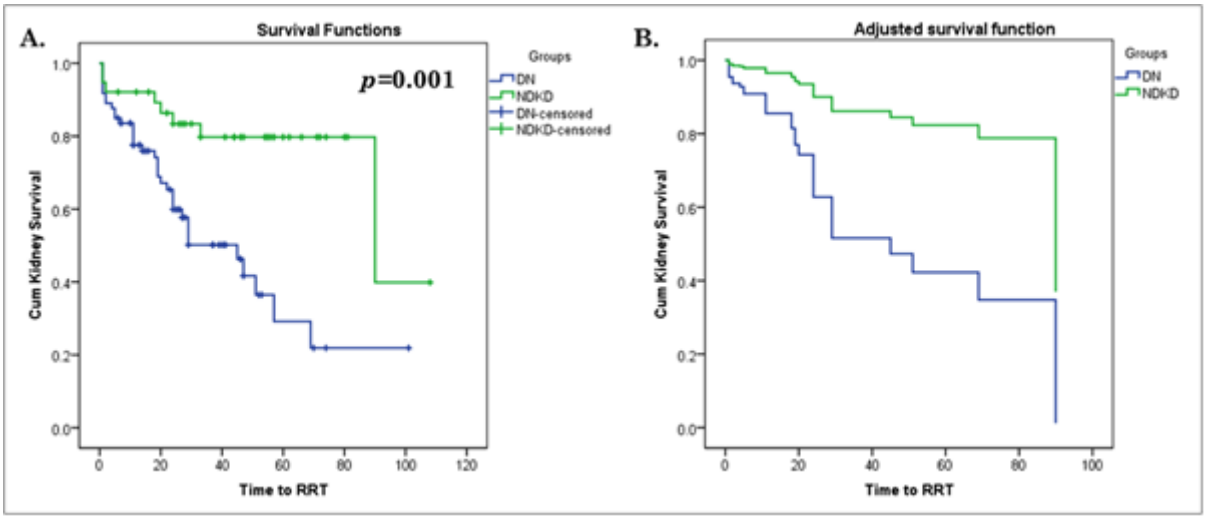


Figure 6. Kidney outcome of enrolled subjects according to biopsy findings: DN (blue line) versus NDKD (green line). a) Event-free survival time (Kaplan-Meier analysis); b) Adjusted event-free survival time. DN: diabetic nephropathy; NDKD: non-diabetic kidney disease; RRT: renal replacement therapy

A total of 69 (38%) patients died during the follow-up period. There were no differences in mortality between the two groups (39% in DN and 37% in NDKD patients, p=0.4).

The kidney survival analysis included the remaining 111 patients (38 with NDKD). RRT was started in 24% of the cohort. More patients in the DN group than in the NDKD group needed RRT initiation: 5 (48%) vs. 8 (21%); p=0.01. The mean kidney survival in the entire cohort was 62 (95%CI 51 to 72) months, and kidney survival at 12, 36 and 60 months was 82, 61 and 52%, respectively.

In univariate time-dependent analysis (**Figure 6a**), patients with NDKD had better kidney survival than those with DN [82 (95%CI 67 to 97) vs. 45 (95%CI 34 to 56.5) months, log rank p=0.001]. Moreover, the kidney survival advantage persisted after adjusting for the risk factors for CKD progression (**Figure 6b**). Beside the histopathologic diagnosis, other independent predictors associated with a poor kidney outcome were eGFR, proteinuria at baseline and absence of treatment with renin-angiotensin system inhibitors.

STUDY 4 – THE PROGNOSTIC ROLE OF DIABETES MELLITUS IN DIABETIC SUBJECTS WITH NON-DIABETIC GLOMERULOPATHIES

In the present study, the prognostic role of diabetes mellitus in adults with non-diabetic glomerulopathies was evaluated, considering the competing risk of death in the kidney survival analysis.

We analyzed all the 1200 subjects selected as previously described in the Research Methodology section.

Primary endpoints of the study were kidney survival and patients' survival.

According to the presence or absence of DM and the types of GP, subjects were divided in three groups: GP without DM (n=987 pts.), GP with DM (n=65 pts.), and diabetic nephropathy (DN) (n=148 pts.). Thirty percent of the subjects in DN group had mixed histologic lesions.

For DN group the main reason for KB was CKD of unknown reason (one third), followed by nephrotic syndrome (almost one third), while for subjects with glomerulopathies other than ND, regardless of the presence of DM diagnosis, the first indication of PBR was nephrotic syndrome, identified more frequently in diabetic patients with GP (half of cases) compared to those without DM (just over a third). The second indication for biopsy for those in the GP with DM group was chronic nephritic syndrome, equal to acute nephritic syndrome (14%), whereas for GP without DM group it was chronic nephritic syndrome (in one-third of subjects).

The main histological pattern of injury among diabetic subjects without DN was membranous nephropathy, followed by IgA nephropathy, compared with non-diabetic subjects in whom the first histological substrate was IgA nephropathy, followed by membranous nephropathy and glomerular nephropathy with minimal damage. Note the small proportion of diabetic patients with lupus nephritis (3%) compared to 11% among non-diabetics.

Vital outcome of subjects with glomerulopathies

During the follow-up period 11% of patients died.

The mortality was higher in diabetic subjects, in similar proportions (18%) in DN and GP with DM groups., but significantly higher in GP without DM group. The survival difference

was also evident in the Kaplan-Meier analysis (log rank p=0,002). The survival was also different between subjects from GP with DM and GP without DM (log rank p=0,04) (**Table 1, Figure 7).**

Table 1. Kidney and overall survival in the three studied groups

Group	No.	RRT YES	Mean survival without RRT (95%CI) months	log rank p	Death YES	Mean survival without death (95%CI) months	log rank p
DN	150	58 (38,7%)	58,1 (46,8-69,5)	<0,001	27 (18%)	95,4 (87,6-103,1)	0,002
GP with DM	63	15 (23,8%)	77,4 (64,2-90,6)		11 (17%)	87,3 (74,5-100)	
GP without DM	987	207 (21%)	94,1 (90,5-97,7)		99 (10%)	110,9 (108,4-113,4)	
All	1200	280 (23,3%)	90,3 (86,9–93,7)		137 (11%)	109 (106,6-111,4)	

DN: diabetic nephropathy; GP: glomerulopathies; DM: diabetes mellitus; RRT: renal replacement therapy.

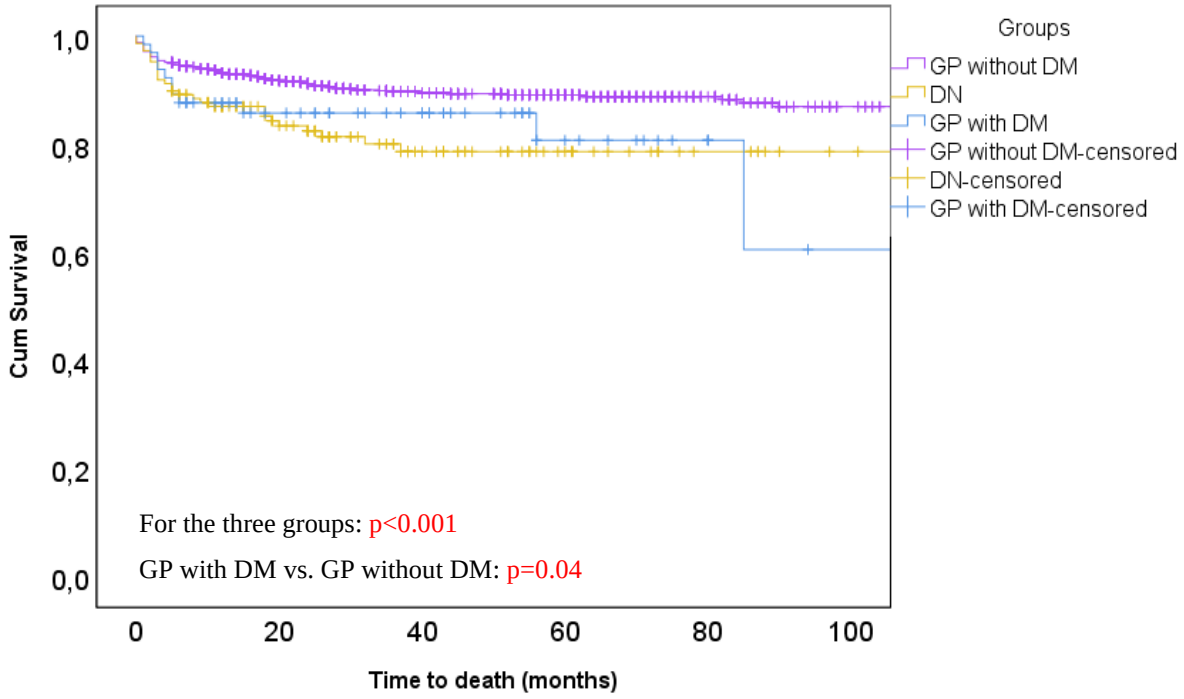


Figure 7. Overall survival in the three analyzed groups. DN: diabetic nephropathy; GP: glomerulopathies; DM: diabetes mellitus.

Two multivariate models were built to identify independent risk factors contributing to death. In the first model, significant comorbidities in the univariate analysis (diabetes mellitus and congestive heart failure) were entered in addition to the other significant parameters. In model 2, only the Charlson comorbidity index was entered as an indicator of disease burden, with diabetes and heart failure already included in the calculation of the Charlson index.

The risk of death was not predicted by history of diabetes (in model 1) or by the type of histological involvement in multivariate analysis (in models 1 and 2). Independent predictors

of mortality were age, presence of congestive heart failure, lower hemoglobin, serum albumin, and serum cholesterol in model 1. In model 2, the Charlson comorbidity index and absence of renin-angiotensin system blockers independently predicted death, in addition to age and lower levels of hemoglobin, albumin and serum cholesterol.

Kidney outcome in subjects with glomerulopathies

The RRT was needed in 23% of all subjects. Kidney survival at 12, 24, 36, 48, and 60 months was 85%, 81%, 77%, 73%, and 68% respectively.

The Kaplan-Meier analysis revealed a difference in kidney survival among the three studied groups, the shortest survival time being recorded in those with ND (**Figure 8, Table 1**). There was no difference in kidney survival between the GP with DM and GP without DM subjects (log rank p=0.1).

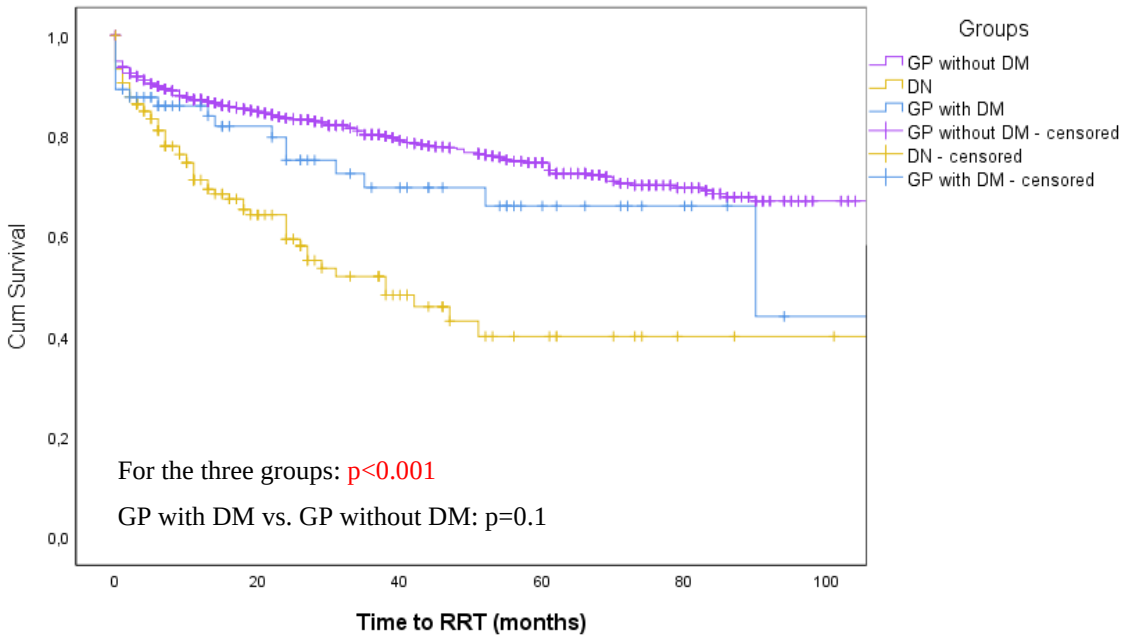
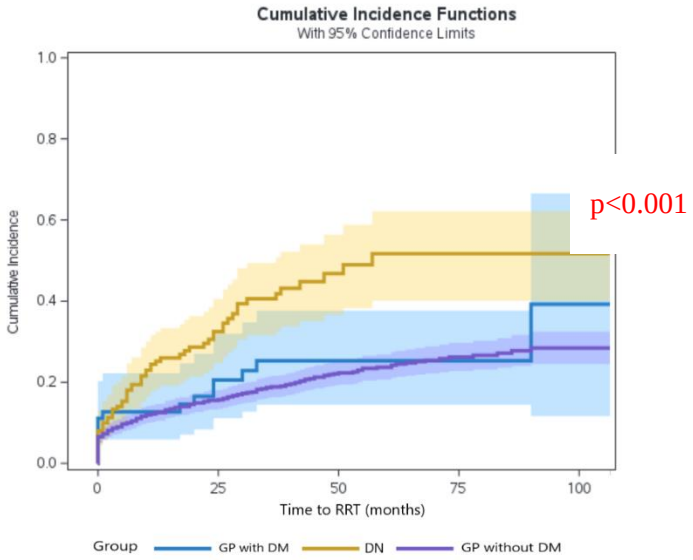


Figure 8. Cumulative survival without RRT in the analyzed groups. DN: diabetic nephropathy; GP: glomerulopathies; DM: diabetes mellitus; RRT: renal replacement therapy.



Group	Event of interest (RRT)	Competing event (death)	Censored	Total
DN	58	27	65	150
GP with DM	15	11	37	63
GP without DM	207	99	681	987
Total	280	137	783	1200

Figure 8. Cumulative incidence of RRT in the analyzed groups considering the risk of death as competing event.
DN: diabetic nephropathy; GP: glomerulopathies; DM: diabetes mellitus; RRT: renal replacement therapy.

In the competitive risk time to event analysis where death was considered the competing event, a significantly difference in kidney survival was observed, sugested by the different cumulative incidence function (CIF) values among the three groups: DN 0.5 (95%CI 0.4-0.6) vs. GP with DM 0.3 (95%CI 0.1-0.6) vs. GP without DM 0.3 (95%CI 0.2-0.4); p<0.001 **(Figure 8**). However, the CIF was not different between GP with DM and GP without DM (p=0.5).

In a subdistribution hazard model in which variables with known effect on CKD progression were entered, diabetes was not maintained as an independent predictor for progression to RRT. Lower eGF, lower serum albumin, and arterial hypertension were independent predictors for dialysis initiation or kidney transplant.

CONCLUSIONS AND PERSONAL CONTRIBUTIONS

Although they are rare conditions, glomerulopathies are the third cause for renal replacement therapy in most countries, including Romania [4]. In addition, diabetic nephropathy - a severe microvascular complication of diabetes characterized by significant histological lesions of the glomeruli, thus also a glomerulopathy, is not included in the reports of the dialysis and kidney transplant registries as glomerulopathy, but in the category of RRT initiated due to diabetes - is the first global cause of RRT. So, cumulatively, glomerulopathies with diabetic nephropathy are the main causes of RRT initiation which, in addition to the significant socio-economic impact, considerably reduces the quality of life and survival of these patients. Thus, using the histological results following native kidney biopsy in adults from the Clinical Hospital of Nephrology "Dr. Carol Davila", a tertiary center that offers nephrological assistance for more than a third of the country's population, a longitudinal, retrospective study was conducted on 1200 adult patients with histologically diagnosed glomerulopathies, with the aim of identifying the most frequent types of glomerular lesions from our country, to describe the characteristics of patients with glomerulopathies and to evaluate the kidney and vital outcome of subjects with these conditions.

The present PhD thesis analyzed the largest group of patients with glomerulopathies in Romania, contributing with important information about the epidemiology of these pathologies, as well as regarding the local medical practice regarding kidney biopsy. For a better picture of the medical practice in our center, we compared ours results with a series of data available from the Norwegian Renal Biopsy Registry: 1866 subjects with KB between 2008 and 2014 in Norway.

Compared to other European countries, including Norway, we draw attention to a significantly lower rate of KB in our center (13.6 pmp/year) – 6.5 times lower than Norway for 2008-2014, respectively 5 times lower for the entire analyzed period (2008-2017); which places us among the last places in Europe, being followed only by Serbia and another center in northwestern Romania.

Like most European and non-European studies, we noted a slightly higher proportion of glomerulopathies diagnosis in men, with the mean age at diagnosis corresponding to the 5th and 6th decades of life.

The main type of glomerulopathy histologically diagnosed in our center was IgA nephropathy, a result consistent with most reports from European countries, including Norway. After IgAN, membranous nephropathy and diabetic nephropathy were the most common types of GP. We observed an important increase in diabetic nephropathy diagnosis in the last years (4.5 times more in the last 3 years of analysis compared to the first 3 years), with a decrease in primitive GP diagnosis (membranous NP, MCD). The most uncommon types of GP were diffuse endocapillary GN and hereditary GP. Men were most often diagnosed with IgAN, followed by membranous nephropathy, while women were more frequently diagnosed with lupus nephritis, followed by IgAN.

The most common indication for KB, in almost half of the cases, was nephrotic syndrome, followed by chronic nephritic syndrome, compared to Norway where the first reason for KB was chronic nephritic syndrome, followed by nephritic-nephrotic syndrome. This finding suggests that the referral to nephrologist in Romania is rather based on symptoms than on more subtle laboratory abnormalities. However, the dynamics of KB indications during the ten years period in our center, revealed a change in medical practice in recent years, by extending the KB indication to older patients, with lower kidney function and less clinically obvious clinical manifestations - CKD of unknown reason, asymptomatic urinary abnormalities; which also led to a change in the frequency of some types of glomerulopathies, in favor of secondary ones (diabetic nephropathy, amyloidosis, crescentic GN).

With the increase in life expectancy, the frequency of elderly with CKD, including glomerulopathies, also increases. The present work brings valuable information regarding the frequency of glomerulopathies among the elderly, the safety of performing KB in this category of patients, as well as about the kidney prognosis of the elderly with GP. In present, there are few studies in the literature addressing this subject. From the results of the second study, which evaluated the kidney prognosis and GP characteristics in the elderly (≥65 years), it appears that the main indications for performing KB in this category of patients were nephrotic syndrome and acute nephritic syndrome, and the most common types of GP histologically diagnosed were membranous nephropathy and amyloidosis. The result of kidney biopsy changed the immunosuppressive therapy indication in almost half of the patients older than 65 years, while the rate of biopsy complications was low. Considering these aspects, as well as the fact that the kidney prognosis of patients with GP does not appear to be worse with advancing age, we conclude that kidney biopsy provides essential information for diagnosis, management, and prognosis, and is safe, even in patients over 65 years of age with glomerulopathies.

The present work also addressed the frequency and characteristics of glomerular lesions in subjects with diabetic kidney disease. Diabetic patients represented approximately one fifth of the analyzed cohort, being predominantly middle-aged men with multiple comorbidities. Among these patients, one third were histologically diagnosed with glomerular lesions other than diabetic nephropathy (non-diabetic kidney disease - NDKD).

The results of the third study indicate that the most common causes of NDKD were membranous nephropathy and IgA nephropathy, and in one out of five diabetic patients, the histological result imposed a change in therapy. Thus, we conclude that histological diagnosis in diabetic subjects is essential to clarify the epidemiology of DKD, as well as to optimize the therapeutic strategy.

Furthermore, the histological diagnosis of glomerulopathies in diabetic patients also brings valuable information about prognosis, as it results from the fourth study of the PhD thesis. Thus, although the mortality of patients with diabetes and glomerular lesions other than diabetic nephropathy is higher than that of non-diabetic patients with glomerulopathies, the risk of RRT of patients with non-diabetic GP appears to be similar, regardless of history of diabetes, even when considering the risk of dying before RRT is needed. In contrast, the kidney prognosis of patients with diabetic nephropathy is significantly more severe compared to that of patients with GP, diabetic or not. To our knowledge, this is the first study to evaluate kidney survival in diabetic subjects with glomerulopathies considering the competing risk of death in the occurrence of the kidney event.

Based on the contributions of the current PhD thesis, a nationwide Romanian Kidney Biopsy Register may be founded that would facilitate the analysis of the dynamics of the epidemiology of glomerulopathies and the prognosis of these conditions with the aim of developing early detection and prevention programs so that the medical assistance is improved.

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