



UNIVERSITY OF MEDICINE AND PHARMACY  
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**MORPHOLOGICAL PROGNOSTIC  
FACTORS IN RENAL DAMAGE OF  
SYSTEMIC LUPUS ERITHEMATOSUS  
(SLE)**

**ABSTRACT OF THE PhD THESIS**

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The doctoral thesis contains 48 figures and 39 tables. The abstract includes a limited number of their total, keeping the numbering in the thesis. The selective bibliography presented here includes 44 of the total 411 bibliographic references of the doctoral thesis.

**CHAPTER 4**  
**MORPHOLOGICAL VARIABILITY IN LUPUS NEPHRITIS: DIAGNOSIS CLASSES, SCORES OF  
LESION, ACTIVITY AND CHRONICITY INDEXES, CLINICO-MORPHOLOGICAL  
CORRELATIONS**

**4.1. INTRODUCTION**

Progression of renal damage in systemic lupus erythematosus (SLE) results in a variety of glomerular and interstitial morphological lesions. Renal biopsy is the gold standard for diagnosis. The accumulations of clinical and pathological observations resulted, in 1982, in the first WHO (World Health Organization) Classification of lupus glomerulonephritis (Churg, Sobin, 1982). This classification, appreciated as simple, easy to learn, quickly achievable and reproducible, has become the standard method of diagnostic classification. As a result, renal biopsy has gained its guiding value for establishing therapy in lupus nephritis (Lewis, 1999). Since 2004, the diagnostic criteria introduced by the International Society of Nephrology / Renal Pathology Society (ISP/RPS) (Weening et al., 2004a, Weening et al., 2004b) have been applied to establish the histopathological diagnosis of lupus nephritis. This new classification provides nephropathologists with a more precise characterization of diagnostic classes, with an impact on increasing interobserver reproducibility (Markowitz, D'Agati, 2007, Markowitz, D'Agati, 2009, Giannico, Fogo, 2013). The efforts of the specialists in order to formulate semi-quantitative evaluation criteria for assessing active and / or chronic lesions are worth mentioning. These concerns, initiated in 1964 (Pollak et al., 1964), were materialized in 1983 by the semi-quantitative scoring algorithm proposed by the National Institute of Health (NIH) of the USA (Austin et al., 1983, Austin et al., 1984) which is still used at the moment.

The review of the literature on lupus nephritis in the last 10 years continues to reveal the low level of reproducibility, due to the inconsistencies in defining the histological parameters, which lead to important interobserver variations in the process of lesion evaluation and establishing the diagnostic class (Wilhelmus et al., 2015). Therefore, the need to revise this classification has been a priority in nephropathology. In 2016, a group of experts in the pathology of lupus nephritis initiated the project of reaching a consensus in defining the lesions present in this diagnostic entity (Bajema et al., 2018). This scientific approach has been materialized in the consensus report reviewing the ISN / RPS classification of lupus nephritis (Bajema et al., 2018).

**4.2. OBJECTIVE**

The main objective of our study was to investigate the individual morphological changes identified in lupus nephritis, by reference to the diagnostic classes defined according to the ISN / RPS classification, in correlation with the activity and chronicity level. In addition, the analysis of the relationship between the diagnostic classification and the severity of the histopathological lesions aimed at identifying the clinico-morphological correlations and some potential prognostic morphological factors, which can translate predictive value.

The main objective was supported by secondary objectives, which aimed at:

- designing and applying an algorithm for semi-quantitative evaluation of the corpuscular component, rendered into a score of renal corpuscle lesion;
- designing and applying a semi-quantitative evaluation algorithm of the tubulo-interstitial component, rendered into a score of tubulo-interstitial lesion;
- analysis of the clinico-pathological correlations between the diagnostic classes specific to lupus nephritis and the scores of renal corpuscle and tubulo-interstitial lesion, as well as the activity and chronicity indexes;
- establishing the predictive value of renal corpuscle and tubulo-interstitial lesion scores, as well as activity and chronicity indexes, in relation to paraclinical parameters of reference in lupus nephritis.

**4.3. MATERIAL AND METHOD**

The present study is based on the experience of over 20 years in the diagnosis, treatment and monitoring of renal pathology, and implicitly of lupus nephritis, in the Department of Nephrology and the Department of Pathology – Clinical Hospital „Dr. C. I. Parhon” Iași.

### ***Patients***

The study group initially included 57 patients with clinical and paraclinical characteristics suggestive of lupus nephritis, admitted to the Department of Nephrology – Clinical Hospital "Dr. C. I. Parhon" Iași between 2003-2018. For 53 cases, the pathological exam confirmed the diagnosis of lupus nephropathy. 4 cases were excluded from the study, because the collected tissue fragments did not meet the criteria necessary for microscopic evaluation.

### ***Histopathological examination***

The bioptic fragments were harvested by puncture-renal biopsy on native kidneys, in the Department of Nephrology. The renal biopsy fragments were standardly processed, according to the specific working protocol applied in the histopathology laboratories, for examination in light microscopy and in immunofluorescence. Each case was reassessed by microscopic examination. The personal re-evaluation stage was tripled, in parallel, by the evaluation carried out by the student Mădălina Belibou, under the supervision of Prof. Dr. Irina-Draga Căruntu - within the license thesis entitled: *Operational morphological hallmarks in the diagnosis of lupus glomerulonephritis* (Belibou, 2016), and two other independent pathologists.

### ***Semi-quantitative assessment***

In order to evaluate the lesions presented in the puncture-renal biopsy fragments analyzed, we designed and applied two semi-quantitative evaluation algorithms, the first for the renal corpuscle, the second for the tubulo-interstitial component. For each injury we recorded, in the severity report, a score. The proposed and applied algorithm for the assessment of the renal corpuscle lead to a final score for renal corpuscle lesion (RC\_S), resulted by the summing up of all the individual score values granted for each analyzed parameter, with a maximum value of 33. The tubulo-component evaluation algorithm proposed and applied lead to a final score of tubulo-interstitial lesion (TI\_S), resulted the by summing up of all the individual score values granted for each analyzed parameter, with a maximum value of 18.

The morphological parameters analyzed and evaluated for the score of renal corpuscle lesion (RC\_S), respectively the score of tubulo-interstitial lesion (TI\_S), are presented in detail in the following.

#### ***Renal corpuscle score***

##### ***Glomerular component – quantified parameters***

- total number of renal corpuscles;
- total number of normal renal corpuscles;
- number of damaged renal corpuscles (focal or diffuse lesions):
  - focal lesions – score 1;
  - diffuse lesions – score 2;
- mesangial changes (lesions present in more than 50% RCs from the total number of RCs in the biopsy):
  - no hipercellularity – scor 0; low hipercellularity (minimum 3 cells into a single mesangial area) – score 1; moderate hipercellularity (minimum 3 cells in 2 mesangial areas) – score 2; marked hipercellularity (minimum 3 cells in more than 2 mesangial areas) – score 3;
  - no mesangial proliferation – score 0; low mesangial proliferation (deposits into a single mesangial area) – score 1; moderate mesangial proliferation proliferare mezangială moderată (deposits into 2 mesangial areas) – score 2; marked mesangial proliferation (deposits in more than 2 mesangial areas) – score 3;
  - fibrinoid necrosis – score 3;
  - transformation into sclerosis – score 4;
- endothelial changes:
  - absent – scor 0; accumulation of intracapillary inflammatory cells (leukocytes) – scor 1; endocapillary hipercellularity – score 2; endothelial damage / capillary necrosis – score 3; hyalin trombi – score 3; transformation into sclerosis – score 4;
- membranous changes: thickness with „double contour” or „wire loops” aspect:
  - absent – score 0; present – „double contour” – score 1; present – „wire loops” – score 2; cannot be evaluated, transformation into sclerosis – score 4;
- segmental sclerosis:
  - absent – score 0; present in less that 50% of RCs – score 1; present in more than 50% of RCs – score 2; cannot be evaluated, transformation in global sclerosis – score 3;
- crescent: cellular, fibro-cellular, fibrillar:
  - absent – score 0; cellular crescent – score 1; fibro-cellular crescent – score 2; fibrillar crescent– score 3;

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- global sclerosis / glomerulosclerosis:
    - total number of sclerosing RCs;
    - absent – score 0; present in less than 50% of RCs – score 1; present in more than 50% of RCs - score 2; present in more than 75% of RCs - score 3; present in 90-100% of RCs - score 4.
  - Tubulo-interstitial score*
  - Tubulo-interstitial component – quantified parameters*
  - *tubular atrophy*:
    - absent – score 0; present in less than 25% of biopsy area – score 1; present in 25-50% of biopsy area – score 2; present in 50-75% of biopsy area – score 3; present in more than 75% of biopsy area – score 4;
  - dilated tubules:
    - absent – score 0; present – score 1;
  - interstitial chronic inflammatory infiltrate:
    - absent – score 0; present, mild represented (less than 25% of biopsy area) – score 1; present, moderate represented (in 25-50%) – score 2; present, well represented (in 50-75% of biopsy area) – score 3; present, very well represented (more than 75% of biopsy area) – score 4;
  - interstitial acute inflammatory infiltrate:
    - absent – score 0; present – score 1;
  - interstitial fibrosis:
    - absent – score 0; present, mild represented (less than 25% of biopsy area) – score 1; present, moderate represented (in 25-50% of biopsy area) – score 2; present, well represented (in 50-75% of biopsy area) – score 3; present, very well represented (more than 75% of biopsy area) – score 4;
  - vascular lesions (hyalinosis, intimal fibrosis):
    - absent – score 0; fibrinoid necrosis – score 1; intimal fibrosis – score 1; hyalinosis – score 2;
- In parallel, each case was evaluated as activity and chronicity by applying the indexes implemented by the semi-quantitative scoring algorithm proposed by NIH (Austin et al., 1983).

### ***Clinical and paraclinical data***

For the patients included in the study group, we analyzed, in parallel with the histopathological profile, the main demographic and clinico-paraclinical characteristics (urea, creatinine, glomerular filtration rate (GFR) (CKD-EPI), cholesterol, triglycerides, proteinuria within 24 hours, protein / creatinine ratio, hemoglobin, leukocytes, platelets, VSH, anti-cDNA antibodies, anti-phospholipid antibodies, complement C3 fraction) that characterized lupus nephritis. These data were extracted from the patient records. Of the 53 patients, 44 were female, 9 were male, with an average age of  $35 \pm 13$  years.

### ***Statistical analysis***

All clinical, biological and morphological data were stored in a standard database, Excel. Statistical retrieval was performed using SPSS17 software. Numerical values were expressed as means  $\pm$  DS. Pearson correlation and T-test were used to analyze the relationship between the variables considered. The comparisons between groups were evaluated by ANOVA multivariate analysis. For the predictive value we applied the ROC (Receiver Operating Characteristic) analysis and the evaluation of the area under the curve (AUC).

### ***Particularities of organization in the presentation and analysis of results***

Starting from the diagnostic classification of the cases in the diagnostic classes and subclasses of the lupus nephritis, based on the number of cases corresponding to each diagnostic entity and the related histopathological features, we decided to consider:

- for class II and class III: the grouped analysis of all the cases classified in the two classes;
- for class IV: the grouped analysis of all the cases classified in class IV, without reference to subclasses;
- for class V:
  - the grouped analysis of all the cases classified in class V and class V associated with class III / IV, defined as compact class V (evaluation 1);
  - separate analysis of cases classified in class V and respectively in class V associated with class III / IV, defined as separate class V (evaluation 2);
- for class VI: analysis of all the cases included in this class.

This organization, which founded the doctoral study conducted, has as a starting point the complexity of the ISN / RPS classification of lupus nephritis in 6 main classes. We focused our attention on class V, which includes pure class V - defined as membranous lupus nephritis, and class

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V associated with active class III or class IV lesions - considered mixed proliferative lupus nephritis, for which both diagnoses must be reported. We decided to carry out two assessments in parallel, which would allow a thorough analysis of the histological profile that characterizes this diagnostic class. Two arguments were at the basis of this decision, namely: (i) the important differences between the evolution and prognosis of the pure class V and the class V associated with class III / IV, (ii) the limited data existing in the literature, which document, by comparison, the type and the extent of the lesions that make the difference within class V (between subclasses), as well as between class V (compact and separate) and the other diagnostic classes.

### 4.4. RESULTS

#### 4.4.1. HISTOPATHOLOGICAL PICTURE OF LUPUS NEPHRITIS - DIAGNOSTIC CLASSES

Table 4.2. summarizes the diagnostic classification in the lupus nephritis classes, according to the ISN / RPS classification criteria.

**Table 4.2.** Diagnostic classification of cases of lupus nephritis

DIAGNOSIS	# cases
<b><i>Mesangial proliferative lupus nephritis – class II</i></b>	<b>2</b>
<b><i>Focal lupus nephritis – class III</i></b>	<b>4</b>
▪ Class III (A/C) Focal lupus nephritis with active and chronic lesions	2
▪ Class III (A/C) Focal lupus nephritis with chronic lesions	2
<b><i>Diffuse lupus nephritis – class IV</i></b>	<b>19</b>
▪ Class IV-S (A) Diffuse segmental lupus nephritis with active lesions	1
▪ Class IV-S (A/C) Diffuse segmental lupus nephritis with active and chronic lesions	2
▪ Class IV-G (A) Diffuse global lupus nephritis with active lesions	1
▪ Class IV-G (A/C) Diffuse global lupus nephritis with active and chronic lesions	14
▪ Class IV-G (C) Diffuse global lupus nephritis with chronic lesions	1
<b><i>Membranous lupus nephritis – class V</i></b>	<b>22</b>
▪ Class V Membranous lupus nephritis	14
▪ Class V Membranous lupus nephritis associated with	
Class IV-S (A/C) Diffuse segmental lupus nephritis with active and chronic lesions	2
▪ Class V Membranous lupus nephritis associated with	
Class IV-G (A/C) Diffuse global lupus nephritis with active and chronic lesions	6
<b><i>Advanced sclerotic lupus nephritis – class VI</i></b>	<b>6</b>

#### 4.4.2. SEMI-QUANTITATIVE ASSESSMENT OF RENAL CORPUSCLE MORPHOLOGICAL CHANGES - SCORES OF RENAL CORPUSCLE LESION

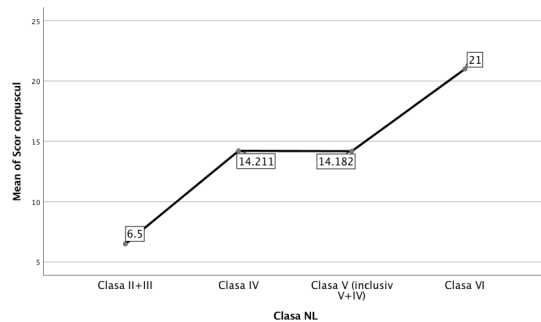
The semi-quantitative analysis of the lesions defined for the glomerular component by the proposed and applied algorithm was materialized by obtaining an RC\_S value for each case.

Regarding the diagnostic classification, the average values of the RC\_S were initially calculated considering the group of class II with class III and, respectively, the group of class V with class V associated with class III / IV. This assessment revealed (Fig. 4.7.): the obvious increase of RC\_S in the sequence of diagnosis severity, from 6.5 for class II + III to 21 for class VI; the average RC\_S values for class IV and class V (including V + IV) are almost similar (14.2 versus 14.18).

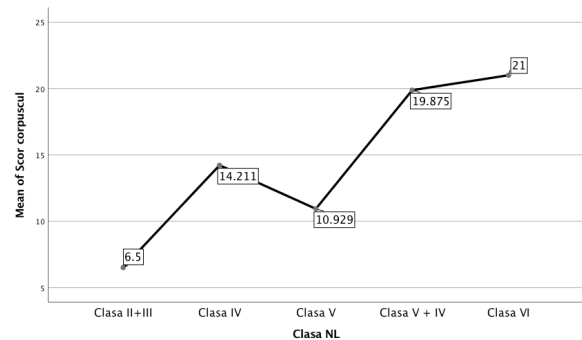
The second method of assessment separated the cases diagnosed as class V from the cases diagnosed as class V associated with class IV. Specifically, we recorded the decrease of the average RC\_S from class IV (14.21) to the class V (10.92), followed by the marked increase of the RC\_S for the class V + IV (19.87), to the small difference from the average RC\_S for class VI (21) (Fig. 4.8.).

*Due to the differences between assessment 1 and assessment 2, for RC\_S, our results indicated the need for a reconsideration of the classification, repositioning class V + IV as a distinct entity in relation to severity, and not as a component of class V.*

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**Fig. 4.7.** RC\_S – valori medii mean values related to the diagnostic classes of lupus nephritis - evaluation 1

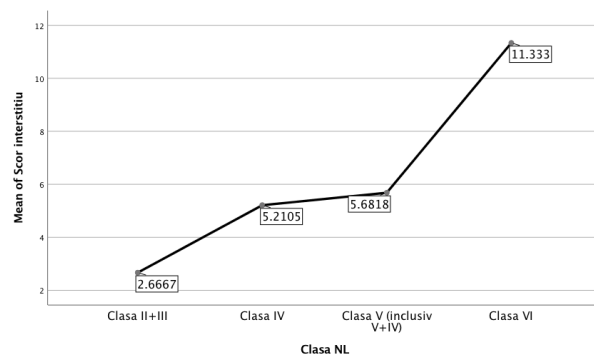


**Fig. 4.8.** RC\_S – mean values related to the diagnostic classes of lupus nephritis - evaluation 2

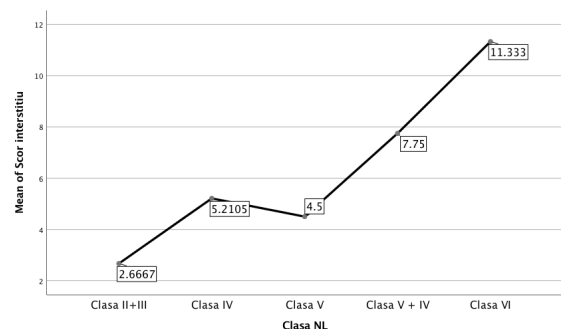
### 4.4.3. SEMI-QUANTITATIVE ASSESSMENT OF TUBULO-INTERSTITIAL MORPHOLOGICAL CHANGES - SCORES OF TUBULO-INTERSTITIAL LESION

The semi-quantitative analysis of the lesions defined for the tubulo-interstitial component by the proposed and applied algorithm was materialized by obtaining a tubulo-interstitial lesion score (TI\_S) value for each case. Regarding the diagnostic classification, the average values of TI\_S were initially calculated considering the group of class II with class III and, respectively, the group of class V with class V associated with class IV.

Our results showed (Fig. 4.9): an obvious increase of TI\_S in the diagnostic severity sequence, from 2.66 for class II + III to 11.33 for class VI; the average values of TI\_S for class IV and class V (including V + IV) very close – 5.21, respectively 5.68.



**Fig. 4.9.** TI\_S – mean values related to the diagnostic classes of lupus nephritis – evaluation 1



**Fig. 4.10.** TI\_S – mean values reported for the diagnostic classes of lupus nephritis – evaluation 2

The second evaluation, considering separately the cases diagnosed as class V from the cases diagnosed as class V associated with class IV, showed the increase of TI\_S from class II + III (2.66) to class IV (5.21), the decrease of TI\_S from class IV to class V (4.5), followed by the increase of TI\_S for class V + IV (7.75), much smaller compared to the average TI\_S for class VI (11.33) (Fig. 4.10).

*Due to the differences between evaluation 1 and evaluation 2, for TI\_S, our results indicated the need for a reconsideration of the classification, repositioning class V + IV as a distinct entity in relation to severity, and not as a component of class V.*

### 4.4.4. SEMI-QUANTITATIVE ASSESSMENT OF ACTIVITY AND CHRONICITY STATUS

The activity index (A\_I) and the chronicity index (C\_I), respectively, showed great variability not only between the diagnostic classes and subclasses, but also within the same class or subclass.

#### *Activity index*

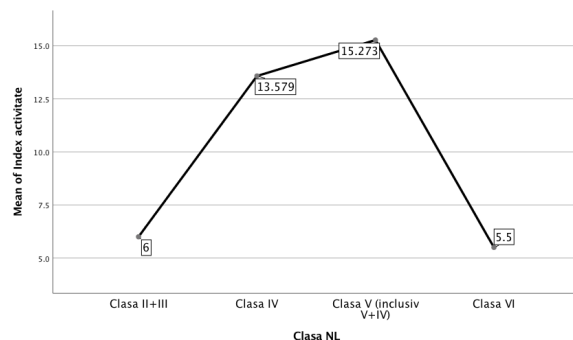
Regarding the diagnostic classification, the average values of A\_I were initially calculated considering the group of class II with class III and, respectively, the group of class V with class V associated with class IV. This evaluation highlighted (Fig. 4.11.):



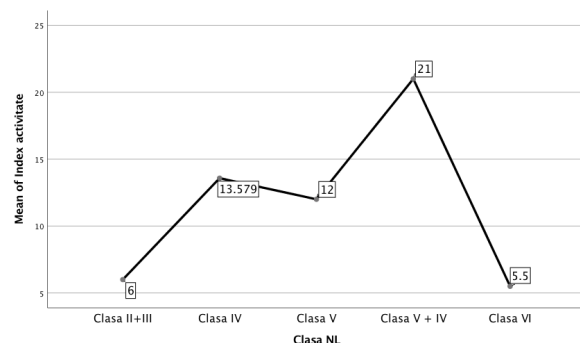
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- the obvious increase of the active status of the lesions identified from class II + III (mean value 6) to class IV (mean value 13.57), followed by a modest increase to class V (including V + IV) (mean value 15.27) and a dramatic decrease in class VI (average value 5.5);
- a small difference, in the actual value of the figures, between the average A\_I for class IV and class V (including V + IV) – 13.57 versus 15.27.

For the second evaluation, we applied the same pattern as for RC\_S and TI\_S, considering separately diagnosed cases as class V from cases diagnosed in class V associated with class IV. By separating class V from class V + IV, we registered the decrease of A\_I from class IV (13.57) to class V (12), followed by the increase of A\_I for class V + IV (21), with the dramatic decrease for class VI (5.5) (Fig. 4.12).



**Fig. 4.11.** A\_I – mean values related to the diagnostic classes of lupus nephritis - evaluation 1



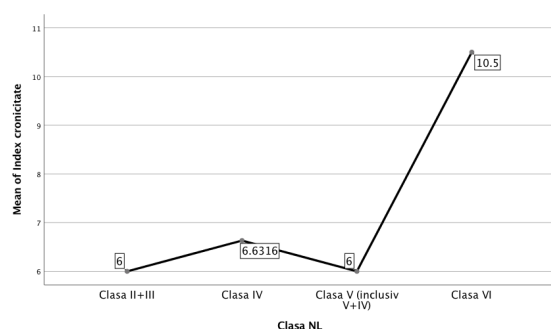
**Fig. 4.12.** A\_I – mean values related to the diagnostic classes of lupus nephritis - evaluation 2

### *Chronicity index*

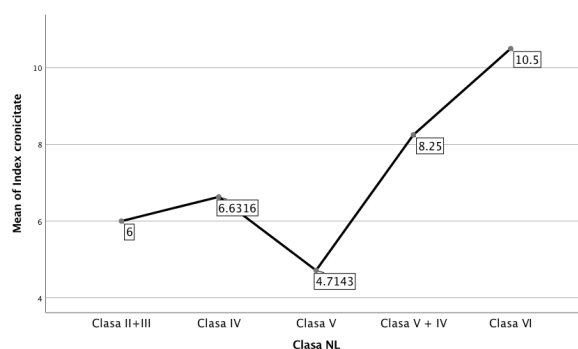
Regarding the diagnostic classification, the average values of C\_I were calculated initially considering the group of class II with class III and respectively the group of class V with class V associated with class III / IV. This evaluation highlighted (Fig. 4.13):

- an obvious increase in the chronic status of the lesions identified from classes II + III, IV and V (including V + IV) (mean value 6, respectively 6.63, respectively 6) to class VI (mean value 10.5);
- minimum differences between class II+III, class IV and class V (including class V associated with class IV) - 6 versus 6.63 versus 6.

The data obtained revealed an important similarity in the development and progression of chronic lesions in lupus nephritis class II, class III, class IV and class V (including class V associated with class IV), although each class constitutes a distinct diagnostic entity in the RPS/ISP Classification.



**Fig. 4.13.** C\_I – mean values reported in the diagnostic classes of lupus nephritis – evaluation 1



**Fig. 4.14.** C\_I – mean values reported in the diagnostic classes of lupus nephritis – evaluation 1

The second evaluation was performed by separating the cases diagnosed in class V from the cases diagnosed in class V associated with class IV. We noted the decrease of C\_I from class II+III (6) and class IV (6.63) to class V (4.71), followed by the increase of C\_I for class V + IV (8.25), and the consecutive increase to class VI (10.5) (Fig. 4.14).

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Due to the differences between assessment 1 and evaluation 2, for A\_I and C\_I, our results indicated the need to separate the two subclasses, V and V + IV, respectively, for a realistic assessment of the evolution and prognosis, as well as the need for a reconsideration of the classification in order to transpose class V + IV as a distinct entity in relation to gravity, and not as a component of class V.

## 4.4.5. SYNOPSIS OF SEMI-QUANTITATIVE ASSESSMENTS OF RENAL CORPUSCLE AND TUBULO-INTERSTITIAL MORPHOLOGICAL CHANGES AND ACTIVITY AND CHRONICITY STATUS

The semi-quantitative analysis based on the application of RC\_S and TI\_S and, respectively, of the activity and chronicity indexes led to an integrated and comparative picture, at the same time, of the degree of severity of the histopathological changes that characterize each case.

The individual results obtained were reported for each class and subclass for diagnosis of lupus nephritis.

## 4.4.6. CLINICO-MORPHOLOGICAL CORRELATIONS IN LUPUS NEPHRITIS. VARIABILITY OF RENAL CORPUSCLE AND TUBULO-INTERSTITIAL LESION SCORES, AND ACTIVITY AND CHRONICITY INDEXES, IN RELATION TO DIAGNOSTIC CLASSES

For the patients included in the studied group, we analyzed, in parallel with the histopathological profile, the main demographic and clinico-paraclinic characteristics, which underpin the picture of lupus nephritis.

The ANOVA multivariate analysis performed, considering the compact class V (class V associated with class IV), revealed the presence of statistically significant differences between the diagnostic classes of lupus nephritis for creatinine values ( $p = 0.001$ ), GFR (CKD-EPI) ( $p = 0.046$ ), triglycerides ( $p = 0.016$ ) and complement C3 fraction ( $p = 0,031$ ) (Table 4.8.).

Similar results were obtained in ANOVA multivariate analysis, which included, separately, the diagnostic class V and the diagnostic class V+IV, with statistically significant differences between the considered lupus nephritis classes, for creatinine values ( $p = 0.003$ ), GFR (CKD-EPI) ( $p = 0.066$ ), triglycerides ( $p = 0.036$ ) and complement C3 fraction ( $p = 0,024$ ) (Table 4.9.).

**Table 4.8.** Demographic and paraclinical characteristics related to the diagnostic classes of lupus nephritis - evaluation 1

PARAMETERS	TOTAL (N=53)	CLASS II+III (N=6)	CLASS IV (N=19)	CLASS V (N=22)	CLASS VI (N=6)	p*
Age (years)	35±13	32±9	38±16	35±12	31±11	0.619
Urea (mg%)	89.89±69.66	41.75±14.59	88.5±78.79	95.33±72.3	110±44.99	0.495
Creatinine (mg%)	1.69±1.79	1.03±0.77	1.2±0.78	1.54±1.36	4.45±3.56	0.001
GFR (ml/min)	68.18±40.84	75.01±41.45	72.98±36.24	74.1±43.87	24.44±14.75	0.046
Cholesterol (mg%)	230.67±79.61	204.2±48.68	239.08±59.06	243.05±97.55	174.5±50.24	0.376
Triglyceride (mg%)	199.44±76.13	141±20.45	195.54±58.28	231.84±85.68	131.25±21.7	0.016
Proteinuria 24 (g/24h)	15.53±46.97	1.7±1.94	5.43±5.9	30.08±72.33	12.45±19.6	0.458
Protein / creatinine ratio	4.9±4.75	2.86±1.27	5.55±4.57	5.48±5.38	1.45±1.3	0.372
Hemoglobin (g%)	10.15±2.22	12.03±1.47	10.25±2.23	9.84±2.09	9.6±3	0.317
Leukocyte (mm <sup>3</sup> )	7246.07±4719.9	5023.5±2303.9	8659.4±5635.5	6434.7±3746.7	7626.1±6157.6	0.392
Platelet (mm3)	239100±114678.1	247500±108445.07	246647±141811.03	217080.9±93667.4	299200±103608.4	0.537
VSH (mm/2h)	68.27±41.06	51.8±29.84	58.81±39.29	82.3±38.71	56.5±62.47	0.232
Anti-cDNA antibodies	209.49±157.01	118.88±134.38	223.77±129.52	249.98±177.47	117.2±123.8	0.197
Anti-phospholipid antibodies	16.17±10.36	12.88±10.84	22.3±12.75	14.28±8.26	0±0	0.398
C3	62.23±26.78	80.25±35.56	54.27±19.73	55.95±21.02	91.25±36.85	0.031

\* ANOVA

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**Table 4.9.** Demographic and paraclinical characteristics related to the diagnostic classes of lupus nephritis - evaluation 2

PARAMETERS	Total (N=53)	Class II+III (N=6)	Class IV (N=19)	Class V (N=14)	Class V+IV (N=8)	Class VI (N=6)	p*
Age (years)	35±13	32±9	38±16	37±11	32±12	31±11	0.653
Urea (mg%)	89.89±69.66	41.75±14.59	88.5±78.79	69±56.38	138.12±78.14	110±44.99	0.113
Creatinine (mg%)	1.69±1.79	1.03±0.77	1.2±0.78	1.38±1.39	1.83±1.34	4.45±3.56	0.003
GFR (ml / min)	68.18±40.84	75.01±41.45	72.98±36.24	79.97±42.65	63.83±46.98	24.44±14.75	0.066
Cholesterol (mg%)	230.67±79.61	204.2±48.68	239.08±59.06	259.23±107.8	213±72.62	174.5±50.24	0.327
Triglyceride (mg%)	199.44±76.13	141±20.45	195.54±58.28	235.17±91.75	226.14±80.81	131.25±21.7	0.036
Proteinuria 24h (g / 24h)	15.53±46.97	1.7±1.94	5.43±5.9	39.15±82.3	2.89±2	12.45±19.6	0.357
Protein / Creatinine ratio	4.9±4.75	2.86±1.27	5.55±4.57	6.05±6.28	4.43±3.31	1.45±1.3	0.459
Hemoglobin (g%)	10.15±2.22	12.03±1.47	10.25±2.23	10.42±2.26	8.91±1.46	9.6±3	0.211
Leukocyte (mm <sup>3</sup> )	7246±4719.9	5023.5±2303.9	8659.4±5635.5	6896.9±3912	5683.8±3582.2	7626.1±6157.6	0.51
Platelets (mm <sup>3</sup> )	239100±114678.1	247500±108445	246647±141811	248384.6±86028.6	166212.5±87195	299200±103608.4	0.317
VSH (mm / 2h)	68.27±41.06	51.8±29.84	58.81±39.29	70.85±37.37	103.57±33.75	56.5±62.47	0.119
Anti-cDNA antibodies	209.49±157.01	118.88±134.38	223.77±129.52	190.9±165.71	342.7±164.42	117.2±123.8	0.06
Anti-phospholipid antibodies	16.17±10.36	12.88±10.84	22.3±12.75	14.9±10.59	13.05±0.07	0±0	0.616
C3	62.23±26.78	80.25±35.56	54.27±19.73	63.22±19.32	43.83±19.29	91.25±36.85	0.024

\* ANOVA

The ANOVA multivariate analysis of the average values of RC\_S and respectively TI\_S, as well as of A\_I and C\_I, in the evaluation type of compact V class, revealed statistically significant differences between the diagnostic classes of lupus nephritis for RC\_S (p <0.001), TI\_S (p <0.001) and A\_I (p = 0.001). No statistically significant differences were obtained for C\_I (Table 4.10.).

On the other hand, in the conditions in which we considered separate class V - respectively class V and class V+IV, we obtained statistically significant differences between all the diagnostic classes of lupus nephritis, for all 4 instruments of semi-quantitative evaluation - including for C\_I (p <0.001, p = 0.022) (Table 4.11.).

**Table 4.10.** Semi-quantitative evaluation tools related to the diagnostic classes of lupus nephritis - evaluation 1

SEMI-QUANTITATIVE ASSESSMENT	CLASS II+III (N=6)	CLASS IV (N=19)	CLASS V (N=22)	CLASS VI (N=6)	TOTAL (N=53)	p*
RC_S	6.5±2.51	14.21±3.28	14.18±6.69	21±0.63	14.09±5.88	<0.001
TI_S	2.67±2.42	5.21±2.42	5.68±3.96	11.33±1.75	5.81±3.74	<0.001
A_I	6±4.65	13.58±4.4	15.27±8.17	5.5±3.99	12.51±7.18	0.001
C_I	6±4.65	6.63±2.93	6±4.44	10.5±1.64	6.74±3.9	0.081

\* ANOVA

**Table 4.11.** Semi-quantitative evaluation tools related to the diagnostic classes of lupus nephritis - evaluation 2

SEMI-QUANTITATIVE ASSESSMENT	CLASS II+III (N=6)	CLASS IV (N=19)	CLASS V (N=14)	CLASS V+IV (N=8)	CLASS VI (N=6)	TOTAL (N=53)	p*
RC_S	6.5±2.51	14.21±3.28	10.93±5.14	19.88±5.19	21±0.63	14.09±5.88	<0.001
TI_S	2.67±2.42	5.21±2.42	4.5±4	7.75±3.11	11.33±1.75	5.81±3.74	<0.001
A_I	6±4.65	13.58±4.4	12±8.07	21±4.54	5.5±3.99	12.51±7.18	<0.001
C_I	6±4.65	6.63±2.93	4.71±4.66	8.25±3.11	10.5±1.64	6.74±3.9	0.022

\* ANOVA

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To refine the understanding of the way the instruments proposed by the present doctoral study (RC\_S, TI\_S), as well as the classical instruments (A\_I, C\_I), are effective in the semi-quantitative evaluation of lupus nephritis lesions, we statistically analyzed the relation between the score / index values established for a diagnostic class and the score / index values of each of the other diagnostic classes. The obtained results varied according to the classification of cases in class V, in evaluation alternative 1, compact (all cases diagnosed in class V and in class V+IV) (Table 4.12), and in evaluation alternative 2, with separation of class V of class V+IV (Table 4.13). The index of chronicity was an exception from these differences, for which there were no statistically significant differences.

**Table 4.12.** Statistically significant differences ( $p < 0.05$ , ANOVA – comparison between groups) between RC\_S, TI\_S, A\_I, C\_I, according to the lupus nephritis class - evaluation 1

SIGNIFICANT DIFFERENCES	CLASS II+III	CLASS IV	CLASS V TOTAL	CLASS VI
RC S	IV, V, VI	II+III, VI	II+III, VI	II+III, IV, V
TI S	VI	VI	VI	II+III, IV, V
A I	V	NU	II+III, VI	V
C I	No	No	No	No

**Table 4.13.** Statistically significant differences ( $p < 0.05$ , ANOVA – comparison between groups) between RC\_S, TI\_S, A\_I, C\_I, according to the lupus nephritis class - evaluation 2

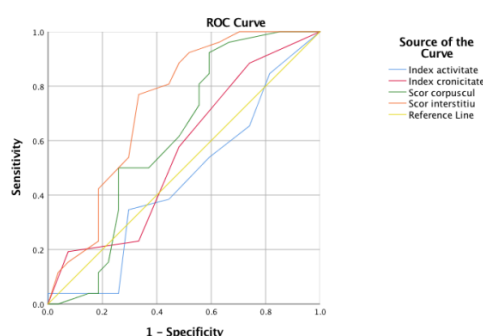
SIGNIFICANT DIFFERENCES	CLASS II+III	CLASS IV	CLASS V	CLASS V+IV	CLASS VI
RC S	IV, V+IV, VI	II+III, V+IV, VI	V+IV, VI	II+III, IV, V	II+III, IV, V
TI S	V+IV, VI	VI	VI	II+III	II+III, IV, V
A I	V+IV	V+IV, VI	V+IV	II+III, IV, V, VI	IV, V+IV
C I	No	No	VI	No	V

## 4.4.7. CLINICO-MORPHOLOGICAL CORRELATIONS IN LUPUS NEPHRITIS. PREDICTIVE POWER OF THE HISTOLOGICAL LESIONS IN RELATION TO THE CLINICO-BIOLOGICAL PARAMETERS

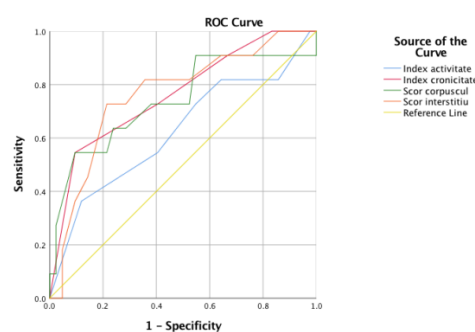
The ROC analysis for RC\_S and TI\_S, respectively A\_I and C\_I and the clinico-paraclinical parameters considered relevant for the specific renal impairment of lupus nephritis allowed *the study of the predictive value of these semi-quantitative instruments, in the sense of their predictive power for the severity of the renal impairment at the time of evaluation by renal biopsy.*

Consecutively, the ROC curves and the evaluation of AUC revealed:

- a good prediction for an GFR  $< 60$  ml / min (the clinical diagnosis threshold of stage III chronic kidney disease) in the addressing moment (AUC 0.732,  $p = 0.004$ ) in relation to TI\_S (fig. 4.17.);
- a good prediction for a GFR  $< 30$  ml / min in the addressing moment (clinical diagnosis threshold of stage IV chronic kidney disease), provided by RC\_S (AUC 0.740,  $p = 0.015$ ), TI\_S (AUC 0.763,  $p = 0.008$ ), and C\_I (0.763  $p = 0.008$ ) (fig. 4.18.);
- absence of predictive power for a proteinuria  $> 3.5$ g / 24h (nephrotic proteinuria) in the addressing moment or at the point of renal biopsy, in relation to both scores (AUC 0.491,  $p = 0.915$ , respectively AUC 0.528,  $p = 0.722$ ) and both indexes (AUC 0.511,  $p = 0.887$ , respectively AUC 0.452,  $p = 0.545$ ) (fig. 4.19.).

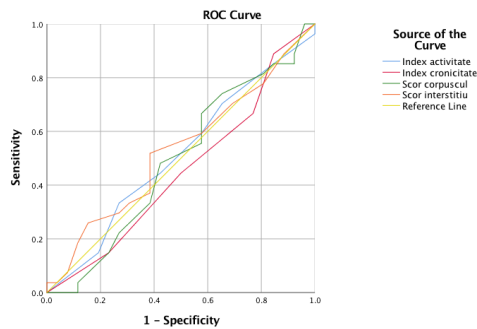


**Fig. 4.17.** ROC curve for GFR  $< 60$  ml/min at addressing



**Fig. 4.18.** ROC curve for GFR  $< 30$  ml/min at addressing

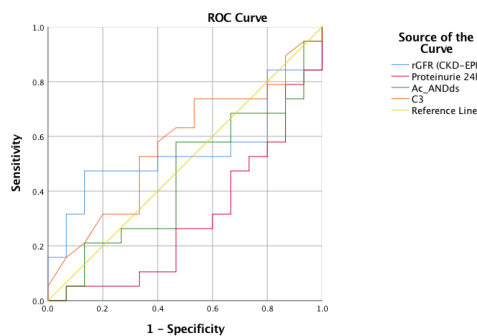
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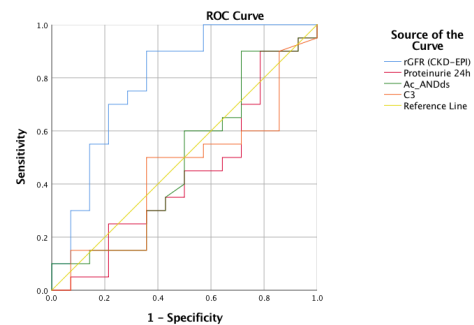
**Fig. 4.19.** ROC curve for proteinuria > 3.5g/24h

### 4.4.8. CLINICO-MORPHOLOGICAL CORRELATIONS IN LUPUS NEPHRITIS. PREDICTIVE POWER OF THE CLINICO-BIOLOGICAL PARAMETERS IN RELATION TO THE HISTOLOGICAL LESIONS

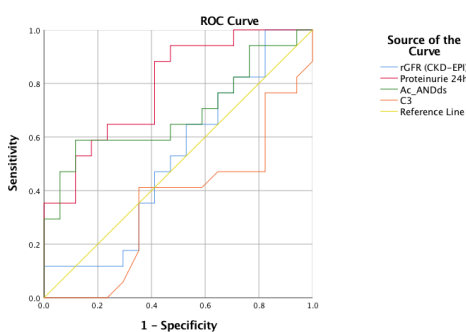
The results obtained in the analysis of the predictive power of the histological lesions in relation to the clinico-biological parameters led to the formulation of a point of view that brings to the center the clinical picture of the patient. Consecutively, we analyzed *the predictive value of some paraclinical parameters in the addressing moment - as a reflection of the pathogenic mechanism and the consequent morphological changes.*



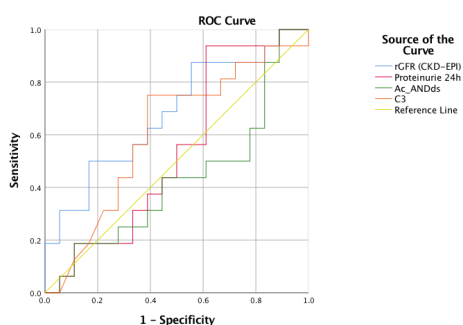
**Fig. 4.20.** ROC curve: RC\_S over median as a predictor for GFR, proteinuria / 24h, anti-cDNA and C3 antibodies



**Fig. 4.21.** ROC curve: TI\_S over median as predictor for GFR, proteinuria / 24h, anti-cDNA and C3 antibodies



**Fig. 4.22.** ROC curve: A\_I above median as a predictor for GFR, proteinuria / 24h, anti-cDNA and C3 antibodies



**Fig. 4.23.** ROC curve: C\_I above median as predictor for GFR, proteinuria / 24h, anti-cDNA and C3 antibodies

The ROC curve and the AUC evaluation for GFR, proteinuria, anti-cDNA and C3 antibodies, in predicting an above average RC\_S in the addressing moment illustrated a poor prediction for all paraclinical parameters analyzed (Fig. 4.20.).

The ROC curve and the AUC for GFR, proteinuria, anti-cDNA and C3 antibodies, in predicting an above-average TI\_S in the addressing moment illustrated a good prediction for GFR (AUC 0.793,  $p = 0.004$ ) (Fig. 4.21.).

The ROC curve and the AUC for GFR, proteinuria, anti-cDNA and C3 antibodies, in predicting an A\_I above the median in the addressing moment illustrated a good prediction for proteinuria / 24h (AUC 0.789,  $p = 0.004$ ) (Fig. 4.22.).

The ROC curve and the AUC for GFR, proteinuria, anti-cDNA and C3 antibodies, in predicting an C\_I above the median in the addressing moment illustrated a poor prediction for all paraclinical parameters analyzed (Fig. 4.23.).

## **4.5. DISCUSSIONS**

### **4.5.1. THE VALUE OF RENAL BIOPSY - HISTORICAL HALLMARKS AND MODERN TRENDS**

The overview into the history of this technique supports its value, emphasized through the association with the progresses made in histopathology, immunology, immunopathology, biochemistry and genetics and through the compulsory integration into the clinical context of the patient. The molecular and genetic perspectives bring added value to diagnosis, mainly in hereditary renal pathology (Reeve et al., 2009). However, the histopathological examination represents the turning point in the classification of renal diseases, providing the major marks for the morphological and pathogenic background.

### **4.5.2. THE VALUE OF QUALITATIVE AND SEMI-QUANTITATIVE EVALUATION OF MORPHOLOGICAL CHANGES IN LUPUS NEPHRITIS**

#### *4.5.2.1. Hallmarks in the evolution of the quantification of renal morphological lesions*

The ISN / RPS classification includes elements of differentiation between active and chronic status, based on the morphology of the renal corpuscle (Weening et al., 2004a, Weening et al., 2004b). Correspondingly, the reporting of activity and chronicity indexes (Austin et al., 1983), as indicators of the potential for reversibility or irreversibility of the lesions, provides additional information on the relation between the lesion background and the differentiated therapeutic response (Wang et al., 2012).

#### *4.5.2.2. Review of ISP / RPS Classification - working principles, progress, limits, perspectives, reporting on personal results*

The review of the literature on lupus nephritis reveals a high variability in the interpretation and the reporting of the lesions that lead to classification. Therefore, the interobserver consensus rate is low due to the inconsistencies existing in the ISP / RPS classification (Weening et al., 2004a, Weening et al., 2004b). Based on this reality, in 2016 a group of experts in the pathology of lupus nephritis initiated the revision of the ISP / RPS classification, following the proposal of an updated classification, with a higher degree of applicability and reproducibility (Bajema et al., 2018). The objective was to achieve a consensus in defining the lesions that are present in lupus nephritis (Bajema et al., 2018). As a working methodology, the group decided to formulate two types of recommendations. The *Phase 1 recommendations*, which are implementable, targeted the morphological elements that could be clarified and defined precisely, by the consensus of the participants in the meeting. The *Phase 2 recommendations* are recommendations for future research, which target lesions that are still debatable in interpretation and for which evidence-based approach are still needed.

*Considering the article published in Kidney International in 2018 (Bajema et al., 2018), the current PhD thesis follows an actual and relevant framework. The starting idea of the doctoral study was to refine the level of interpretation of the identified lesions, in order to be able to correlate them with the intensity of the disease. Thus, our evaluation algorithm, built before the publication of the article, and our results support the value of the study.*

### **4.5.3. PREDICTIVE VALUE OF RENAL CORPUSCLE AND TUBULO-INTERSTITIAL LESION SCORES, AND ACTIVITY AND CHRONICITY INDEXES**

The review of the literature emphasizes the interest to establish correlations between the clinical picture of lupus nephritis and the histopathological background present in the renal corpuscles and the interstitial component, with repercussions in the ability to anticipate the course and prognosis (Schwartz et al., 2008). Although the studies dedicated to this subject are not very numerous, they support the predictive value for certain histopathological parameters, in correspondence with the clinical classification. The difficulty of this approach, however, results from a multitude of factors: the large differences between the design of the studies (including the characteristics of the studied population, the dimensions of the study groups, the selection criteria, the analyzed diagnostic class or

classes, the clinical, paraclinical and morphological parameters investigated), the interobserver variability in the assessment of histopathological changes and, finally, the changes in the classification of lupus nephritis (Singh et al., 2011).

*Starting from the data present in the mainstream, our study is individualized by two elements. The first element is represented by the differentiated approach, in analysis, of the class V of lupus nephritis. Thus, in order to be able to appreciate the value of the class versus subclass diagnostic classification, we considered class V without subclasses (hereinafter referred to as compact class V), and respectively class V in the variant of the two subclasses (which we will refer to as a separate class V). The second original element of the present study consists in adding the reference to the two proposed scores – RC\_S and TI\_S, to the classic semi-quantitative evaluation system that includes A\_I and C\_I.*

*Our results reveal statistically significant differences between the classes of lupus nephritis and serum creatinine, FGR (CKD-EPI), triglycerides and the C3 fraction of the complement, in both variants of statistical analysis, which included compact class V and separate class V.*

*Our data revealed a different picture of the renal corpuscle and tubulo-interstitial lesions, as well as of the activity and chronicity status, in the compact class V and the separate class V. However, in relation to the paraclinical, biological parameters, the doctoral study indicates a similar behavior of the subclasses classified in the class V of lupus nephritis. This finding is an argument in favor of the proposal to abandon subclass diagnosis in lupus nephritis (Hill et al., 2005, Sada et al., 2009, Haring et al., 2012), motivated by the lack of relevance of the subclasses in the prognostic (Hill et al., 2005, Sada et al., 2009, Haring et al., 2012, Satish et al., 2017).*

The doctoral study provides solid, additional evidence in favor of the significance of evaluating the pathological background of lesions specific to lupus nephritis, especially since the identified changes have predictive value. Our results complement the literature data discussed above, according to which the A\_I and the C\_I provide consistent information for assessing evolution and prognosis (Jacobsen et al., 1999, Bihl et al., 2006, Shariati-Sarabi et al., 2013, Satish et al., 2017, Wilson et al., 2018). The interstitial infiltrate (Park et al., 1986, Esdaile et al., 1989, Hill et al., 2000, Hsieh et al., 2011, Wilson et al., 2018) is considered an independent prognostic factor, and the interstitial fibrosis and tubular atrophy are significant predictive factors for renal survival (Wilson et al., 2018).

*In contrast to one of the first studies that analyze the prognostic relevance of histopathological lesions in lupus nephritis, and which confirms the predictive value only for C\_I (Esdaile et al., 1989), our data reveal the analytical significance in assessing the evolution for all 4 tools for semi-quantitative evaluation – two classics (A\_I, C\_I) and two originals (RC\_S, TI\_S). It is also worth noting that the average values obtained by us were higher than those reported in the literature - A\_I  $12.51 \pm 7.18$  versus  $5.48 \pm 4.138$  (Satish et al., 2017), C\_I  $6.74 \pm 3.9$  versus  $1.52 \pm 2.374$  (Satish et al., 2017), which further highlights interobserver variability in the assessment of lupus nephritis lesions as well as lesion heterogeneity.*

In the present study, the correlations between the diagnostic classes and the instruments of semi-quantitative evaluation of the present lesions highlighted not only the evolving character of the lesions, but also the value of the separation of the class V into subclasses. *These results are an argument for the existence of the two different diagnostic subclasses within class V of lupus nephritis, namely membranous lupus nephritis (class V) and membranous lupus nephritis associated with focal or diffuse lupus nephritis (class III / IV), contrary to the proposal of renunciation to the subclass diagnosis – as mentioned above – which is motivated by data that indicate the absence of prognostic relevance (Hill et al., 2005, Sada et al., 2009, Haring et al., 2012, Satish et al., 2017). However, the results obtained by us offer a different perspective on histopathological differences that reflect lesions of diverse intensity. Consequently, the histological evaluation, qualitative and semi-quantitative, provides the clinician access to refined information, whose applicability in the prognostic evaluation is worth taking into consideration.*

The histopathological picture allows an analysis focused on the pathogenic mechanism of lupus nephritis. The morphological significance is amplified at the level of each diagnostic entity, because the identified renal lesions reinforce the pathophysiological sequence, in the following order: (i) the formation of antigen-antibody complexes; (ii) the morphologically objectified renal injury. The intensity of the lupus nephritis specific immune response correlates directly with the severity of the microscopic lesions. The diagnostic classification in class V must be analyzed in relation to two different pathogenic mechanisms. Class V defined as pure membranous glomerulonephritis is characterized by the presence



of immune complexes in the subepithelial space, away from the vascular space – a situation in which the activation of the complement system by the classical pathway does not end with influx of inflammatory cells; consequently, the lesions are located at the level of the glomerular basement membrane. On the other hand, class V includes the diagnostic category membranous glomerulonephritis associated with diffuse proliferative glomerulopathy – class V+IV. These cases reflect a pathogenic mechanism that includes localization of subepithelial immune deposits, affecting the function of the glomerular basement membrane and podocytes (specific for class V), but also the localization of subendothelial and mesangial immune deposits, impacting the cell proliferation (specific for class IV). *Consequently, the statistically significant differences recorded in the analysis of the relationship between the score / index values established for a diagnostic class and the score / index values of each of the other diagnostic classes, depending on the classification of the cases in the compact class V (class V and class V+IV) and respectively the separate class V (class V versus class V+IV) can be explained in the pathophysiological context that sustains the initiation and development of morphological lesions.*

*One of the major objectives of our study consists of the predictive potential of the clinico-morphological picture present in lupus nephritis. Our analysis shows the predictive value of the histological lesions, translated by the values of the scores and indexes, as follows: TI\_S – for the prediction of a GFR <60 ml / min, as an indicator for the stage III chronic kidney disease; RC\_S, TI\_S, C\_I – for the prediction of an GFR <30 ml / min in the addressing moment, as indicator for the stage IV chronic kidney disease in the addressing moment. None of the two scores and two indexes indicated a predictive power for a nephrotic proteinuria (> 3.5g/24h) in the addressing moment. On the other hand, our analysis supports the predictive value of some clinical parameters, as follows: GFR – for the prediction of a TI\_S over median; proteinuria – for the prediction of an A\_I.*

Last but not least, we underline that the recent literature indicates inconsistencies between the clinical picture and the histological picture of lupus nephritis, mainly the proliferative forms (Malvar et al., 2017). Therefore, the prognostic assessment and the therapeutic decision must be based on a complex, integrated evaluation of the clinico-morphological picture. In this context, the histopathological information provided by the proposed (RC\_S, TI\_S) and operational (A\_I, C\_I) semi-quantitative instruments has a manifest significance, offering concrete indicators for constructing a personalized monitoring plan.

*In summary, the originality of our study consists in proposing a paraclinico-histological phenotype of the gravity that can characterize lupus nephritis. This phenotype includes two categories of criteria:*

- *histologically, TI\_S, whose values may indicate stage III or stage IV chronic kidney disease, together with RC\_S and C\_I, whose values may indicate stage IV chronic kidney disease;*
- *clinically, GFR, whose values may indicate an TI\_S above the median, and proteinuria whose values may indicate an A\_I above the median.*

## **CHAPTER 5**

### **LYMPHOCYTE POPULATIONS IN LUPUS NEPHRITIS: BETWEEN PATHOGENESIS AND PROGNOSTIC FACTORS**

#### **5.1. INTRODUCTION**

Immunologically, lupus nephritis is considered a prototype for autoimmune pathology characteristic of chronic diseases due to the deposition of immune complexes, with the consecutive activation of the complement in the classical and alternative ways (Wągrowaska-Danilewicz, Danilewicz, 2014). The autoimmune component recognized in the pathogenic mechanism involves the abnormal activity of B lymphocyte (B Ly), which becomes self-reactive, as well as the formation of abnormal activated T lymphocytes (T Ly), resulting in the production of autoantibodies, immune complexes and cytokines that determine the development of an inflammatory response (Choi et al., 2012, Mohan, Putterman, 2015). The repetitive sequences of the inflammatory response influence the chronic course of the disease.

A review of the literature focusing on the role of lymphocytes in the pathogenic mechanism of lupus nephritis reveals that studies targeting the profile of human lymphocyte populations are limited, most of them being from experimental research. B Ly intervenes in the pathogenesis of lupus nephritis



through signaling abnormalities and failure in immunological tolerance. Studies in murine models have revealed abnormal signaling mechanisms through B Ly, the AKT / mTOR pathway playing a primary role (Wu et al., 2007). T Ly are the most common inflammatory cells identified in renal injury in both patients with SLE and in murine models of lupus nephritis (D'Agati et al., 1986, Diaz Gallo et al., 1992, Couzi et al., 2007, Deng et al., 2010). There is an intense discussion about the correlation between the composition of the inflammatory infiltrate and the severity of the tubulo-interstitial impairment, resulting in the progression of renal damage and the risk of installing the terminal renal failure (Alexopoulos et al., 1990, Yu et al., 2010, Hsieh et al., 2011). T Ly present in the renal interstitium are predominantly CD4+, CD28+, without memory, and CD8+.

## **5.2. OBJECTIVE**

The main objective of our study was to analyze the T Ly population profile associated with lupus nephritis lesions. The motivation underlining this objective was supported by the fact that the data regarding the involvement of T Ly in the pathogenesis of lupus nephritis, in human subjects, are limited. The main objective included two secondary objectives, which aimed at:

- qualitative and quantitative characterization of the population of CD4+ and CD8+ T Ly present in different classes of lupus nephritis, in the periglomerular, intraglomerular and interstitial territories;
- correlation of the quantitative profile of CD4+ T Ly and CD8+ T Ly with RC\_S, TI\_S, A\_I and C\_I.

## **5.3. MATERIAL AND METHOD**

### ***Patients***

The study group analyzed was similar to the one presented in Chapter 5, section 5.2. Material and method - respectively 53 patients, diagnosed histopathologically with lupus nephritis and treated in the Department of Nephrology – Clinical Hospital “Dr. C.I. Parhon” Iași, between 2003-2018. In presenting and analyzing the results we applied the same organizational features, detailed in section 5.2. Material and method. The material used was the paraffin blocks corresponding to the renal tissue fragments taken by puncture-renal biopsy, previously diagnosed histopathologically as lupus nephritis, which were processed for IHC examination.

### ***Immunohistochemical examination***

The IHC staining for identifying antigens complementary to anti-CD4+ and anti-CD8+ antibodies (Table 5.1.) was performed using the BenchMark XT (Ventana Medical System, Inc., Tucson, AZ) automatic staining system.

### ***Quantitative assessment***

For the quantitative evaluation of CD4+ and CD8+ T Ly we used a score reported in the literature (Hsieh et al., 2011), adapted to the histological features of renal parenchyma. CD4+ and CD8+ T Ly were quantified in three distinct territories: periglomerular (CD4\_PG, CD8\_PG), intraglomerular (CD4\_IG, CD8\_IG) and interstitial (CD4\_IT, CD8\_IT). The immunohistochemically marked cells were counted using a magnification of 400X. In each case and each territory, the results were expressed as the number of positive, immunoreactive cells per square millimeter.

### ***Statistical analysis***

Statistical analysis was performed using SPSS17 software. Numerical values were expressed as means  $\pm$  DS. Pearson correlation and T-test were used to analyze the relationship between the considered variables. Comparisons between groups were performed using the Mann-Whitney U non-parametric test and ANOVA multivariate analysis.

## **5.4. RESULTS**

### **5.4.1. HISTOPATHOLOGICAL CHARACTERISTICS OF THE STUDY GROUP - SYNOPSIS**

The morphological characterization of the lesions accomplished in the doctoral study through the semi-quantitative evaluation based on RC\_S, TI\_S, A\_I and C\_I provided the following mean values for the lupus nephritis classes:

- RC\_S: evaluation 1: 6.5 for class II+III, 14.2 for class IV, 14.18 for class V (including V+IV), 21 for class VI; evaluation 2: 6.5 for class II+III, 14.2 for class IV, 10.929 for class V, 19.875 for class V+IV, 21 for class VI.
- TI\_S: evaluation 1: 2.66 for class II+III, 5.21 for class IV, 5.68 for class V (including V+IV), 21

for class VI; evaluation 2: 2.66 for class II+III, 5.21 for class IV, 4.5 for class V, 7.75 for class V+IV, 11.33 for class VI.

- A\_I: evaluation 1: 6 for class II+III, 13.57 for class IV, 15.27 for class V (including V+IV), 5.5 for class VI; evaluation 2: 6 for class II+III, 13.57 for class IV, 12 for class V, 21 for class V+IV, 5.5 for class VI.
- C\_I: evaluation 1: 6 for class II+III, 6.63 for class IV, 6 for class V (including V+IV), 10.5 for class VI; evaluation 2: 6 for class II+III, 6.63 for class IV, 4.71 for class V, 8.25 for class V+IV, 10.5 for class VI.

#### **5.4.2. THE PROFILE OF CD4+ T LYMPHOCYTES**

##### ***Qualitative evaluation***

Qualitative analysis revealed, in relation to the renal corpuscles, the predominantly periglomerular arrangement of the CD4+ T Ly, very few cells being present at intraglomerular level, in the lumen of the glomerular capillaries or in the mesangium; CD4+ T Ly were present in the interstitium, among the tubular structures, with a generally diffuse organization.

##### ***Quantitative evaluation***

Within the lot as a whole, the average number of CD4+ T Ly was  $111 \pm 78.40 / \text{mm}^2$  of renal biopsy. In the different territories considered in relation to lupus nephritis lesions, the mean values quantified for CD4+ T Ly revealed the net predominance of interstitial CD4+ T Ly ( $\text{mean}_{IT} = 70.24 \pm 44.87 / \text{mm}^2$ ) versus periglomerular CD4+ T Ly ( $\text{mean}_{PG} = 37.69 \pm 39.49 / \text{mm}^2$ ) versus intraglomerular CD4+ T Ly ( $\text{mean}_{IG} = 3.05 \pm 3.27 / \text{mm}^2$ ).

In relation to the lupus nephritis diagnostic classes, we obtained the following values of the average number of CD4+ T Ly:

- Lupus nephritis – class II+III (6 cases): mean value was  $81 \pm 56.56 / \text{mm}^2$ , with the following distribution:  $32.67 \pm 10.52 / \text{mm}^2$  at periglomerular level,  $1.5 \pm 1.05 / \text{mm}^2$  at intraglomerular level and  $59.17 \pm 23.63 / \text{mm}^2$  at interstitial level, as a result of the grouped evaluation for:
  - Mesangial proliferative lupus nephritis – class II (2 cases):  $81 \pm 56.56 / \text{mm}^2$ , with the following distribution:  $20.5 \pm 4.94 / \text{mm}^2$  at periglomerular level,  $1 \pm 1.41 / \text{mm}^2$  at intraglomerular level and  $59.5 \pm 50.20 / \text{mm}^2$  at interstitial level;
  - Focal lupus nephritis – class III (4 cases):  $99.5 \pm 13.12 / \text{mm}^2$ , with the following distribution:  $38.75 \pm 5.31 / \text{mm}^2$  at periglomerular level,  $1.75 \pm 0.95 / \text{mm}^2$  at intraglomerular level and  $59 \pm 9.48 / \text{mm}^2$  at interstitial level;
- Diffuse lupus nephritis – class IV (19 cases, of which 3 cases class IV-S and 16 cases class IV-G):  $110.05 \pm 42.37 / \text{mm}^2$ , with the following distribution:  $33.31 \pm 13.11 / \text{mm}^2$  at periglomerular level, only  $3.15 \pm 3.60 / \text{mm}^2$  at intraglomerular level and  $73.57 \pm 35.25 / \text{mm}^2$  at interstitial level; the mean numerical values of CD4+ T Ly were very close in the two diagnostic subclasses, respectively  $113 \pm 44.69 / \text{mm}^2$  in class IV-G vs  $94.33 \pm 27.06 / \text{mm}^2$  in class IV-S;
- Membranous lupus nephritis – class V, class V+IV (22 cases):  $90.31 \pm 40.83 / \text{mm}^2$ , with the following distribution:  $30.77 \pm 14.7 / \text{mm}^2$  at periglomerular level, only  $3.77 \pm 3.58 / \text{mm}^2$  at intraglomerular level and  $55.77 \pm 25.55 / \text{mm}^2$  at interstitial level, as a result of the grouped evaluation for:
  - Membranous lupus nephritis – class V (14 cases):  $77.5 \pm 41.18 / \text{mm}^2$ , with the following distribution:  $24.92 \pm 12.79 / \text{mm}^2$  at periglomerular level,  $2.57 \pm 2.92 / \text{mm}^2$  at intraglomerular level and  $50 \pm 28.35 / \text{mm}^2$  at interstitial level;
  - Membranous lupus nephritis – class V associated with diffuse lupus nephritis – class IV (8 cases):  $112.75 \pm 29.01 / \text{mm}^2$ , with the following distribution:  $41 \pm 12.50 / \text{mm}^2$  at periglomerular level,  $5.87 \pm 3.83 / \text{mm}^2$  at intraglomerular level and  $65.87 \pm 16.78 / \text{mm}^2$  at interstitial level;
- Advanced sclerotic lupus nephritis – class VI (6 cases):  $207.5 \pm 190.63 / \text{mm}^2$ , with the following distribution:  $82 \pm 109.15 / \text{mm}^2$  at periglomerular level, only  $1.67 \pm 1.51 / \text{mm}^2$  at intraglomerular level and  $123.83 \pm 92.79 / \text{mm}^2$  at interstitial level.

##### ***Clinico-pathological correlations***

In the overall assessment of the entire study group, considering the values obtained for all 53 cases analyzed by quantification at the level of the three relevant territories, the statistical analysis (Mann-Whitney test) revealed statistically significant differences between CD4+ T Ly located at intra, periglomerular and interstitial levels ( $p < 0.0001$ ).

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Statistical analysis of the quantitative profile of CD4+ T Ly (T-test) between diagnostic classes compared in the classification dynamics that reflects the severity of the lesions (class II *versus* class III, class III *versus* class IV, class IV *versus* class V, class V *versus* class V+IV, class V *versus* class VI), for each of the 3 territories of interest, highlighted the following statistically significant differences (Table 5.5.):

- between periglomerular CD4+ T Ly ( $p = 0.0156$ ), in class II *versus* class III;
- between interstitial CD4+ T Ly ( $p = 0.0482$ ), in class IV *versus* class V;
- between periglomerular CD4+ T Ly ( $p = 0.0097$ ) and intraglomerular CD4+ T Ly ( $p = 0.0338$ ), in class V *versus* class V+IV;
- between interstitial CD4+ T Ly ( $p = 0.0610$ ), in class V *versus* class VI.

We noted the absence of statistically significant differences between CD4+ T Ly quantified in class III *versus* class IV, and class IV-G *versus* class IV-S, in periglomerular, intraglomerular and interstitial territories.

**Table 5.5.** Significant statistical differences in the quantitative profile of the CD4+ T Ly, with regard to the diagnostic class and relevant territories

DIAGNOSTIC CLASS (t test)	CD4+ T lymphocytes/mm <sup>2</sup>		
	PG	IG	IT
II vs III	$p = 0.0156$	$p = 0.4689$	$p = 0.9836$
III vs IV	$p = 0.4305$	$p = 0.4562$	$p = 0.4289$
IV vs V	$p = 0.0760$	$p = 0.6246$	$p = 0.0486$
V vs V+IV	$p = 0.0097$	$p = 0.0338$	$p = 0.1162$
V vs VI	$p = 0.0610$	$p = 0.4831$	$p = 0.0125$

In parallel, the ANOVA multivariate analysis performed between all diagnostic classes, in evaluation 1 (compact class V – class V together with class V+IV) and in evaluation 2 (class V separated in class V and class V+IV respectively) revealed statistically significant differences between periglomerular CD4+ T Ly and interstitial CD4+ T Ly (Table 5.6., Table 5.7.).

**Table 5.6.** Statistically significant differences in the quantitative profile of the CD4+ T Ly, between all diagnostic classes and the territories accounted for – evaluation 1

CD4+ T LYMPHOCYTES	Class II+III (N=6)	Class IV (N=19)	Class V total (N=22)	Class VI (N=6)	p (ANOVA)
CD4_PG	32.67±10.52	33.32±13.12	30.77±14.7	82±109.15	$0.031$
CD4_IG	1.5±1.05	3.16±3.61	3.77±3.58	1.67±1.51	0.328
CD4_IT	59.17±23.63	73.58±35.26	55.77±25.55	123.83±92.79	$0.007$

**Table 5.7.** Statistically significant differences in the quantitative profile of the CD4+ T Ly, between all diagnostic classes and the territories accounted for – evaluation 2

CD4+ T LYMPHOCYTES	Class II+III (N=6)	Class IV (N=19)	Class V pure (N=14)	Class V+IV (N=8)	Class VI (N=6)	p (ANOVA)
CD4_PG	32.67±10.52	33.32±13.12	24.93±12.8	41±12.5	82±109.15	$0.045$
CD4_IG	1.5±1.05	3.16±3.61	2.57±2.93	5.88±3.83	1.67±1.51	0.063
CD4_IT	59.17±23.63	73.58±35.26	50±28.35	65.88±16.79	123.83±92.79	$0.012$

Pearson correlation revealed the absence of significant correlations between RC\_S and CD4+ T Ly present at periglomerular ( $r = 0.116$ ,  $p = 0.2310$ ), intraglomerular ( $r = 0.221$ ,  $p = 0.1113$ ) and interstitial ( $r = 0.223$ ,  $p = 0.1083$ ) levels, as well as between TI\_S and CD4+ T Ly present at periglomerular ( $r = 0.064$ ,  $p = 0.6443$ ), intraglomerular ( $r = 0.192$ ,  $p = 0.1654$ ) and interstitial ( $r = 0.163$ ,  $p = 0.2404$ ) levels. We obtained a significant correlation between A\_I and the number of CD4+ T Ly present only in the intraglomerular area ( $r = 0.330$ ,  $p = 0.0173$ ), the results being without statistical significance for the periglomerular ( $r = 0.226$ ,  $p = 0.1030$ ) and interstitial ( $r = 0.085$ ,  $p = 0.5373$ ) areas. There were no statistically correlations between C\_I and CD4+ T Ly present in the periglomerular ( $r = -0.069$ ,  $p = 0.6177$ ), intraglomerular ( $r = 0.091$ ,  $p = 0.5101$ ) and interstitial ( $r = -0.035$ ,  $p = 0.7984$ ) areas.

### **5.4.3. THE PROFILE OF CD8+ T LYMPHOCYTES**

#### ***Qualitative evaluation***

The qualitative evaluation revealed a higher number of CD8+ T Ly, compared to the population of CD8+ T Ly, with a similar disposition: periglomerular condensation, intraglomerular isolated presence and diffuse agglomeration in the interstitium. Rarely, CD8+ T Ly formed nodular structures, homogeneous or with germinal center (secondary lymphoid nodules).

#### ***Quantitative assessment***

*The CD8+ T Ly population was the best represented subset of lymphocytes. In the study group as a whole, the average number of CD8+ T Ly quantified was  $578.66 \pm 320.53/\text{mm}^2$  of renal biopsy. In the different territories evaluated in relation to lupus nephritis lesions, the mean values quantified for CD8+ T Ly showed a marked predominance of interstitial CD8+ T Ly ( $\text{mean}_{IT} = 372 \pm 206/\text{mm}^2$ ), followed by a significant number of periglomerular CD8+ T Ly ( $\text{mean}_{PG} = 195 \pm 130/\text{mm}^2$ ) and a relatively small number of intraglomerular CD8+ T Ly ( $\text{mean}_{IG} = 11.11 \pm 16.51/\text{mm}^2$ ).*

In the quantitative analysis of the cases relative to the lupus nephritis diagnostic classes, we obtained the following values of the mean number of CD8+ T Ly:

- Lupus nephritis – class II+III (6 cases): mean value was  $195.34 \pm 63.1/\text{mm}^2$ , with the following distribution:  $64 \pm 24.81/\text{mm}^2$  at periglomerular level,  $4.67 \pm 7.63/\text{mm}^2$  at intraglomerular level and  $126.67 \pm 30.66/\text{mm}^2$  at interstitial level, as a result of the grouped evaluation for:
  - Mesangial proliferative lupus nephritis – class II (2 cases): mean value was  $158.5 \pm 23.33/\text{mm}^2$ , with the following distribution:  $65 \pm 15.55/\text{mm}^2$  at periglomerular level,  $1/\text{mm}^2$  at intraglomerular level,  $92.5 \pm 7.77/\text{mm}^2$  at interstitial level;
  - Focal lupus nephritis – class III (4 cases): mean value was  $140.25 \pm 25.25/\text{mm}^2$ , with the following distribution:  $45.25 \pm 23.38/\text{mm}^2$  at periglomerular level,  $6.5 \pm 9.14/\text{mm}^2$  at intraglomerular level,  $88.5 \pm 8.81/\text{mm}^2$  at intestinal level;
- Diffuse lupus nephritis – class IV (19 cases, of which 3 cases IV-S, 16 cases IV-G): mean value was  $481.10 \pm 213.11/\text{mm}^2$ , with the following distribution:  $142.52 \pm 73.97 / \text{mm}^2$  at periglomerular level, only  $15.47 \pm 23.09/\text{mm}^2$  at intraglomerular level and  $323.10 \pm 151.26/\text{mm}^2$  at interstitial level; mean values of Ly T CD8+ were very close in the two diagnostic subclasses considered, respectively  $482.31 \pm 222.64/\text{mm}^2$  in class IV-G versus  $474.66 \pm 192.10/\text{mm}^2$  in class IV-S;
- Membranous lupus nephritis – class V, class V+IV (22 cases): mean value was  $90.31 \pm 43.83/\text{mm}^2$ , with the following distribution:  $30.77 \pm 14.7/\text{mm}^2$  at periglomerular level,  $3.77 \pm 3.58/\text{mm}^2$  at intraglomerular level and  $55.77 \pm 25.55/\text{mm}^2$  at interstitial level, as a result of the grouped evaluation for:
  - Membranous lupus nephritis – class V (14 cases): mean value was  $608.78 \pm 266.25/\text{mm}^2$ , with the following distribution:  $218.71 \pm 130.31/\text{mm}^2$  at periglomerular level,  $8.07 \pm 15.43/\text{mm}^2$  at intraglomerular level,  $382 \pm 169.05/\text{mm}^2$  at interstitial level;
  - Membranous lupus nephritis – class V associated with diffuse lupus nephritis – class IV (8 cases): mean value was  $1001.75 \pm 142.99/\text{mm}^2$ , with the following distribution:  $338.25 \pm 101.44/\text{mm}^2$  at periglomerular level,  $11.37 \pm 5.73/\text{mm}^2$  at intraglomerular level,  $652.12 \pm 88.10/\text{mm}^2$  at intestinal level;
- Advanced sclerotic lupus nephritis – class VI (6 cases): mean value was  $685.5 \pm 358.33 / \text{mm}^2$ , with the distribution:  $260.83 \pm 148.82/\text{mm}^2$  at periglomerular level,  $10.5 \pm 6.56/\text{mm}^2$  at intraglomerular level and  $414.16 \pm 207.60/\text{mm}^2$  at interstitial level.

#### ***Clinico-pathological correlations***

In the overall assessment of the entire study group, taking into consideration the values obtained for all 53 cases analyzed in the quantification performed with regard of the three relevant territories, the statistical analysis (Mann-Whitney test) revealed the existence of statistically significant differences between CD8+ T Ly located at intra-, periglomerular and interstitial levels ( $p < 0.0001$ ).

Comparative statistical analysis of the quantitative profile of CD8+ T Ly (T-test) between the diagnostic classes, according to the classification sequence indicating the lesion severity (class II versus class III, class III versus class IV, class IV versus class V, class V versus class V+IV, class V versus class VI), for each of the 3 relevant territories, highlighted the following statistically significant differences (Table 5.9.):

- between periglomerular CD8+ T Ly ( $p = 0.0182$ ) and intraglomerular CD8+ T Ly ( $p = 0.0062$ ) respectively, in class III versus class IV;

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- between periglomerular CD8+ T Ly ( $p = 0.0411$ ) and interstitial CD8+ T Ly ( $p = 0.0118$ ), in class IV *versus* class V;
- between periglomerular CD8+ T Ly ( $p = 0.0374$ ) and interstitial CD8+ T Ly ( $p = 0.0012$ ), in class V *versus* class V+IV;

We recorded the absence of statistically significant differences between CD8+ T Ly quantified in class II *versus* class III, class IV-G *versus* class IV-S, and class V *versus* class VI, in periglomerular, intraglomerular and interstitial territories.

**Table 5.9.** Statistically significant differences in the quantitative profile of the Ly T CD8+, with regard to diagnostic class and relevant territories

DIAGNOSTIC CLASS	CD8+ T LYMPHOCYTES /mm <sup>2</sup>		
	PG	IG	IT
II <i>vs</i> III	$p = 0.3524$	$p = 0.4673$	$p = 0.6182$
III <i>vs</i> IV	$p = 0.0182$	$p = 0.0062$	$p = 0.4598$
IV <i>vs</i> V	$p = 0.0411$	$p = 0.3071$	$p = 0.0118$
V <i>vs</i> V+IV	$p = 0.0374$	$p = 0.5701$	$p = 0.0012$
V <i>vs</i> VI	$p = 0.5327$	$p = 0.7177$	$p = 0.7194$

In parallel, the ANOVA multivariate analysis performed between all diagnostic classes, in evaluation 1 (considering class V compact – class V together with class V+IV) and in evaluation 2 (considering class V separated in class V and class V+IV respectively), showed statistically significant differences between periglomerular CD8+ T Ly and interstitial CD8+ T Ly (Table 5.10., Table 5.11.).

**Table 5.10.** Statistically significant differences in the quantitative profile of the Ly T CD8+, between all diagnostic classes and the territories accounted for – 1<sup>st</sup> evaluation

CD8+ T LYMPHOCYTES	Class II+III (N=6)	Class IV (N=19)	Class V total (N=22)	Class VI (N=6)	p (ANOVA)
CD8_PG	64±24.81	158.32±91.06	228.91±163.49	260.83±148.83	0.022
CD8_IG	4.67±7.63	15.47±23.09	9.27±12.69	10.5±6.57	0.483
CD8_IT	126.67±30.66	328.37±154.04	421.27±209.96	414.17±207.61	0.006

**Table 5.11.** Statistically significant differences in the quantitative profile of the CD8+ T Ly, between all diagnostic classes and the territories accounted for – 2<sup>nd</sup> evaluation

CD8+ T LYMPHOCYTES	Class II+III (N=6)	Class IV (N=19)	Class V pure (N=14)	Class V+IV (N=8)	Class VI (N=6)	p (ANOVA)
CD8_PG	64±24.81	158.32±91.06	166.43±161.48	338.25±101.44	260.83±148.83	0.001
CD8_IG	4.67±7.63	15.47±23.09	8.07±15.44	11.38±5.73	10.5±6.57	0.62
CD8_IT	126.67±30.66	328.37±154.04	324.86±175.11	590±155.47	414.17±207.61	<0.001

Pearson correlation indicated significant correlations between RC\_S and CD8+ T Ly present at periglomerular ( $r = 0.436$ ,  $p = 0.017$ ), intraglomerular ( $r = 0.341$ ,  $p = 0.0140$ ) and interstitial ( $r = 0.508$ ,  $p = 0.0002$ ) levels, as well as between TI\_S and the number of CD8+ T Ly present at periglomerular ( $r = 0.332$ ,  $p = 0.0166$ ), intraglomerular ( $r = 0.418$ ,  $p = 0.0026$ ) and interstitial ( $r = 0.366$ ,  $p = 0.0083$ ) levels. We also obtained correlations between A\_I and CD8+ T Ly present in the periglomerular ( $r = 0.276$ ,  $p = 0.0463$ ), intraglomerular ( $r = 0.312$ ,  $p = 0.0245$ ) and interstitial ( $r = 0.453$ ,  $p = 0.0011$ ) areas. The correlations between C\_I and CD8+ T Ly subsets present in the periglomerular ( $r = 0.178$ ,  $p = 0.1995$ ), intraglomerular ( $r = 0.123$ ,  $p = 0.4217$ ) and interstitial ( $r = 0.140$ ,  $p = 0.3125$ ) areas were not statistically confirmed.

#### 5.4.4. SIMILARITIES AND DIFFERENCES BETWEEN THE CD4+ T LYMPHOCYTES' PROFILE AND CD8+ T LYMPHOCYTES' PROFILE

*The population of CD4+ T Ly constituted the much less represented subset of lymphocytes in renal biopsies, compared with CD8+ T Ly. In the whole study group, we registered statistically significant differences between the total population of CD4+ T Ly and the total population of CD8+ T Ly, evaluated by the mean number of lymphocytes / mm<sup>2</sup> for each case ( $p < 0.0001$ ).*

We also obtained statistically significant differences ( $p < 0.0001$ ) between CD4+ T Ly and CD8+ T Ly present at periglomerular ( $\text{mean}_{\text{CD4+}_{\text{PG}}} = 37.69 \pm 39.49 / \text{mm}^2$  versus  $\text{mean}_{\text{CD8+}_{\text{PG}}} = 195.32 \pm 129.92 / \text{mm}^2$ ), intraglomerular ( $\text{mean}_{\text{CD4+}_{\text{IG}}} = 3.05 \pm 3.27 / \text{mm}^2$  versus  $\text{mean}_{\text{CD8+}_{\text{IG}}} = 11.11 \pm 16.51 / \text{mm}^2$ ) and interstitial ( $\text{mean}_{\text{CD4+}_{\text{IT}}} = 70.24 \pm 44.87 / \text{mm}^2$  versus  $\text{mean}_{\text{CD8+}_{\text{IT}}} = 390.15 \pm 271.29 / \text{mm}^2$ ) levels.

The statistically significant differences CD4+ versus CD8+ ( $p < 0.05$ ) were also recorded for all 3 locations, at the level of each diagnostic class, in both assessment modalities – considering class V as being compact (class V and class V+IV) and respectively class V as being separate (class V, class V+IV).

## 5.5. DISCUSSIONS

Our study analyzed the T Ly population profile present in lupus nephritis, observing the association between the T CD8+ (cytotoxic) and T CD4+ (helper) subclasses, in their periglomerular, intraglomerular and interstitial territorial distribution, and the diagnostic classes, in parallel with the activity and chronicity levels of the lesions.

*Our data showed the net predominance of CD8+ T Ly, with statistically significant differences from CD4+ T Ly at global, periglomerular, intraglomerular and interstitial levels, in all diagnostic classes.* This finding is in agreement with the immunological theory that supports the effector role of CD8+ T Ly and that of initiator and target for CD4+ T Ly (Castellino et al., 2006). Furthermore, the ability of a distinct CD4+ T Ly population to stimulate the appearance of CD8+ T Ly is demonstrated, thus optimizing the immune response by the ability of CD8+ T Ly to express an effector cytotoxic phenotype (Castellino et al., 2006). The data obtained complement the limited number of studies published in the literature, focusing on the role of lymphocytes in the pathogenesis and evolution of lupus nephritis (Blanco et al., 2005, Couzi et al., 2007).

*The quantification of T Ly in the immune-inflammatory infiltrate offers the possibility of analyzing the results within the binome: relationship with the immune pathogenic mechanism – prognostic value.*

The differences in the periglomerular, intraglomerular and interstitial localization of the CD8+ and CD4+ T Ly support the particular ability to influence the glomerular lesions on the one hand, and interstitial lesions on the other. This observation is in agreement with the hypothesis that glomerulonephritis lesions are the consequence of systemic autoimmune processes, whereas severe tubulointerstitial inflammation is associated with local, *in situ* immunological processes (Steinmetz et al., 2008, Chang et al., 2011). *In the whole group, we demonstrated the T Ly localization mainly at the interstitial level, with statistically significant differences between the three types of areas considered (periglomerular, intraglomerular and interstitial) for both the CD8+ and CD4+ T Ly population.*

*With strict reference to the correlations between the size of the CD8+ and CD4+ T Ly populations, and the diagnostic classes of lupus nephritis, we consider of interest the comment of some particularities that clearly differentiate the role of the two populations.*

*The CD8+ T Ly population differs significantly, from a statistical point of view, at the periglomerular and interstitial level, between the diagnostic classes of lupus nephritis III, IV, V, V+IV, but not between the diagnostic classes II and III and, respectively, V and VI. This finding supports a steady acceleration of the immune response that may influence glomerular lesions on the one hand and, on the other, interstitial lesions, from class III to class V. The absence of statistically significant differences between CD8+ T Ly in class II and class III can be interpreted in the light of the low level of cytotoxic immune activity in the early stages. In contrast, the absence of statistically significant differences between class V and class VI indicates a depletion of the evolving immune response, in combination with glomerulosclerosis and extensive interstitial fibrosis that characterize stage VI of lupus nephritis. The absence of statistically significant differences between intraglomerular CD8+ T Ly, between all diagnostic classes, is an additional argument for the pathogenic mechanism of the initial glomerular lesion, different from that of the interstitial injury.*

*The CD4+ T Ly population, on the other hand, presented another numerical dynamic and, consequently, we can appreciate a different behavior. The absence of significant differences between class III and IV may indicate the stability of CD4+ T Ly population, after promoting CD8+ T Ly activation. CD4+ T Ly multiplies again at the interstitial level between class IV, class V and class VI, events that may be associated with increased severity of interstitial lesions. Interestingly, the presence of statistically significant differences between periglomerular and intraglomerular CD4+ T Ly of class V and class V+IV may be correlated with the evolution of glomerular lesions.*

According to the data from the literature, the evaluation of the immune-inflammatory infiltrate in lupus nephritis may indicate pathological processes responsible for irreversible renal injury, and the presence of the interstitial immune-inflammatory infiltrate can be interpreted as a potential prognostic factor (Esdaile et al., 1989, Park et al., 1986, Hill et al., 2000).

*Consecutively, we analyzed the relationship between the number of CD8+ and CD4+ T Ly, RC\_S, TI\_S, A\_I and C\_I. Specifically, we demonstrated that CD8+ T Ly are correlated with lesions developed both at the glomerular level (translated by RC\_S), with interstitial changes (translated by TI\_S), and with the activity level of the lesions (evaluated by A\_I). Very interesting is that the influence of CD8+ Ly is manifested regardless of their location. This assumption is supported by the statistical significance correlations obtained between RC\_S and the size of the CD8+ T Ly population located periglomerular, intraglomerular and interstitial. In parallel, with respect to the interstitial changes, TI\_S correlated significantly with the number of the CD8+ T Ly present at periglomerular, intraglomerular and interstitial levels. In counterbalance with this interrelation, we emphasize the absence of significant correlations between RC\_S and TI\_S, and the population of CD4+ T Ly, in all 3 locations (periglomerular, intraglomerular and interstitial).*

The analysis of the relation between CD4+ and CD8+ T Ly populations and A\_I, respectively C\_I indicates two aspects that can be interpreted from the prognostic significance, based on the dynamics of the pathogenic mechanism.

*The first aspect supports the major intervention of the CD8+ T Ly in defining the activity status of lupus nephritis lesions. Specifically, A\_I was significantly correlated with the CD8+ T Ly population present in the periglomerular, intraglomerular and interstitial areas. The participation of CD4+ T Ly is much more limited, our data indicating only the correlation between A\_I and the number of CD4+ T Ly located at intraglomerular level - which explains the initial lesions, reflected by the endocapillary hypercellularity. These data complement the limited reports in the literature, which target the interrelationship between lymphocyte infiltrate and activity index of lupus nephritis (Esdaile et al., 1989, Park et al., 1986, Hill et al., 2000, Coutzi et al., 2007).*

*The second aspect brings into question the chronicity status of the lesions specific to lupus nephritis, in which the severity of lesions already installed can no longer be associated with the immunological response of lymphocyte populations. The progressive dynamics of the lesions, translated by the presence of fibrous crescents in the corpuscles, presence of tubular atrophy and interstitial fibrosis, inflammatory infiltrate predominantly with monocytes, may explain the absence of significant correlations between C\_I and both CD4+ and CD8+ T Ly subsets, in all three (periglomerular, intraglomerular and interstitial) locations. Thus, we can assess that the reactive potential of T Ly, as critical participants in the immune response, is restricted with the installation of chronic lesions.*

Synthesizing all the previous comments, our study aimed to identify the possible correlations between the histology of the lesions in relation to the lupus nephritis classes and the dynamics of the lymphocyte inflammatory infiltrate, following the demonstration of its prognostic valence. The analysis of our results and their projection in the context of previously published data (relatively few and discordant), in view of the wide gap recorded in the numerical profile of T Ly populations, supports the different prognostic value of CD4+ and CD8+ Ly in the development of glomerular and interstitial lesions.

## **CHAPTER 6 CONCLUSIONS**

1. The proposed semi-quantitative algorithm, based on the score of renal corpuscle lesion and score of tubulo-interstitial lesion, ensures the refinement of the identified lesion assessment and the correlation with the intensity and evolution of the lupus nephritis. The data obtained are comparable with the latest achievements in understanding and monitoring lupus nephritis. The two scores are useful tools for the morphological characterization of lupus nephritis, which complements the information obtained by using activity and chronicity indexes, without diminishing their significance.

2. The statistically significant differences recorded in the analysis of the relationship between the score / index values established for a diagnostic class and the score / index values of each of the other diagnostic classes, depending on the classification of the cases in the compact class V (class V



and class V+IV) and respectively the separate class V (class V *versus* class V+IV) must be interpreted in the pathophysiological context that underlies the initiation and development of morphological lesions. In relation to the paraclinical parameters, our results indicate a similar behavior of the subclasses classified in the lupus nephritis class V. This finding is an argument in favor of the current proposal to renounce the diagnosis of subclasses in lupus nephritis, motivated by the lack of relevance of the subclasses for the prognostic.

3. The histological lesions translated by the values of the scores and indexes have a predictive value, as follows: (i) the score of tubulo-interstitial lesion – for the prediction of a GFR <60 ml / min, as an indicator for the stage III chronic kidney disease; (ii) the score of renal corpuscle lesion, score of tubulo-interstitial lesion and chronicity index – for the prediction of a GFR <30 ml/min at presentation, as an indicator for stage IV chronic disease at presentation.

4. Among the clinical parameters, the predictive value was confirmed as follows: (i) GFR – for the prediction of a score of tubulo-interstitial lesion over the median; (ii) proteinuria – for the prediction of an activity index over the median.

5. The established clinico-morphological correlations lead to the proposal of a paraclinico-histological phenotype of gravity that can characterize lupus nephritis. This phenotype includes two categories of criteria: (i) histological, the score of tubulo-interstitial lesion, the values of which may indicate stage III or stage IV chronic kidney disease, together with score of renal corpuscle lesion and chronicity index, whose values may indicate stage IV chronic kidney disease at presentation; (ii) clinically, GFR, whose values may indicate a score of tubulo-interstitial lesion above median, and proteinuria whose values may indicate an activity index above median.

6. The lymphocytic infiltrate associated with lupus nephritis is predominantly composed of CD8+ T Ly. The CD8+ T Ly population differs significantly from a statistical point of view from the CD4+ T Ly population at global, periglomerular, intraglomerular and interstitial levels, in all diagnostic classes. These differences support the different behavior of the two T Ly populations in the pathogenesis of lupus nephritis.

7. The quantitative profile of the CD8+ T Ly population, in all 3 locations (periglomerular, intraglomerular and interstitial), correlates with the intensity of glomerular (assessed by score of renal corpuscle lesion) and interstitial (assessed by score of tubulo-interstitial lesion) damage, as well as with the level activity of lupus nephritis (evaluated by activity index). These correlations support the prognostic potential of CD8+ T Ly population in the development of lupus nephritis.

8. The quantitative profile of intraglomerular CD4+ T Ly population correlates with the activity level of lupus nephritis (evaluated by activity index). This correlation indicates the intervention of CD4+ T Ly in the development of endocapillary hypercellularity.

9. The absence of significant correlations between both CD4+ and CD8+ T Ly populations, in all 3 locations (periglomerular, intraglomerular and interstitial) and the level of chronicity (assessed by the chronicity index) reflects the limitation of the reactive potential of T Ly with the onset of chronic lesions.

10. The variability of the quantitative profile of CD8+ and CD4+ T Ly populations, analyzed in association with renal corpuscle and tubulo-interstitial lesion scores as well as with activity and chronicity indexes, explains the possibility of the inconsistent relationship between glomerular and tubulo-interstitial lesions.

## **CHAPTER 7 PERSPECTIVES**

The doctoral study offers solid prospects for an integrated, clinical and morphological approach to lupus nephritis, based on the personal results obtained, which support the current revision of the ISN / RPS Classification of lupus nephritis and the need to identify prediction and prognostic factors.

The revision of the ISN/RPS Classification was initiated in 2016 and materialized by the consensus report published in 2018 (Bajema et al., 2018), which clarifies the definitions and modifies the NIH indexes for activity and chronicity. As a result, the system of scores proposed and implemented in the evaluation of lupus nephritis is an extremely useful operational tool, with the potential to solve a major classification problem – namely the low reproducibility in the assessment of



lesions. Reporting to the two proposed scores – the score of renal corpuscle lesion and the score of tubulo-interstitial lesion – can complement the classic semi-quantitative evaluation system that includes the activity index and the chronicity index, contributing to the increase of the reproducibility degree in the evaluation.

On the other hand, the validation of some predictive and prognostic factors based on the histopathological picture is a major goal in nephrology, nephropathology, immunology and rheumatology. In this context, the results obtained in the analysis of the predictive potential of the clinico-morphological picture present in lupus nephritis may represent critical points in the personalized approach of the lupus nephritis cases. In this context, we emphasize the value of proposing a paraclinico-histological phenotype of gravity that can characterize lupus nephritis, which includes two categories of criteria, histological and clinical. Also, the involvement of T Ly infiltrate, with clear differences between the CD8+ and CD4+ T subpopulations, represents an area of scientific interest in which research can and should be extended.

Last but not least, the discussion on the characterization of class V of lupus nephritis, through distinct or cumulative reporting to the subclasses currently existing, remains open. The arguments for the elimination of the subclasses are supported by the variability of the intensity of the lesions, reflected by the statistically significant differences between the values of the scores of renal corpuscle and tubulo-interstitial lesions and of the activity and chronicity indexes. In parallel, however, counter arguments must also be considered, supported by paraclinical parameters that indicate a similar behavior of the subclasses pertaining to Class V of lupus nephritis.

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