

SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PHD)

Evaluation of clinical features of lupus nephritis and prognostic factors of chronic kidney disease in patients with systemic lupus erythematosus

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IMMUNOLOGY

DEBRECEN, 2025

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The PhD Defense takes place at the Lecture Hall of Dermatology Department, Faculty of Medicine, University of Debrecen, 2025.06.11.13:00

1. Introduction

Systemic lupus erythematosus (SLE) is a chronic systemic autoimmune disease that affects multiple organ. One of the most important steps in the pathogenesis is the formation of immunocomplexes and their deposition in various tissues and organs. Immunocomplex deposition in kidneys glomeruli are associated with lupus nephritis (LN), which is considered one of the most severe organ manifestations. The prevalence of LN among SLE patients is about 35%, but according to literature data, it ranges from 12-69% in different populations. As lupus nephritis implies persistent alterations to kidney structures and functions, all patients with lupus nephritis have chronic kidney disease (CKD). Among the clinical signs, the appearance of proteinuria, active urine sediment, decreased renal function, nephrosis and nephritis syndrome indicate glomerular damage. At the same time, not only the glomeruli can be involved, but sometimes interstitial and tubular damages occur, or thrombotic microangiopathy develops, which can also contribute to renal impairment. The degree of proteinuria is influenced by the destruction of podocytes. The gold standard for the diagnosis of LN is the renal biopsy. In all cases, if lupus nephritis is suspected, a renal biopsy is recommended, which helps in the differential diagnosis of other glomerular diseases, as well as determines the patient's treatment and also has a prognostic value. LN is classified into six histological categories, which were classified by the WHO, then revised twice by the ISN/RPS leaving the basic histological types intact. The class IV LN, namely diffuse proliferative glomerulonephritis, is the most common histological type and is considered a poor prognostic factor. The progression of CKD and the development of end-stage renal disease (ESRD) are the most prevalent in this category. The introduction of cyclophosphamide was a milestone in the therapy of LN, which significantly improved the survival of patients. In the last two decades, significant advances have been made in the therapy of LN.

In the last decade, the use of mycophenolate mofetil (MMF) has become more and more common in both induction and maintenance treatments. Targeted therapeutic options have also appeared. Belimumab became a registered drug for the treatment of LN and rituximab can also be used in severe cases. Voclosporin, which is a novel calcineurin inhibitor (CNI), has also been approved for treating LN, and several other targeted therapies are under development. Thanks to all of this, the therapeutic options for LN patients are constantly improving. Significant therapeutic advances have also been made in non-renal SLE. New effective guidelines help the treatments, additionally, a new targeted therapy, anifrolumab, has also been approved for the treatment of SLE. The aim of treatment is low disease activity or remission. The main

therapeutic goal is to achieve remission and to minimize the corticosteroid dose or completely stop using corticosteroids. This way the chronic organ damage may be decreased.

Several working groups consider lupus nephritis to be a negative prognostic factor in lupus. However, the diagnostic and therapeutic options have also changed in recent decades; therefore, our first objective was to examine the occurrence and characteristics of LN among SLE patients who were diagnosed after 1990 and received a regular follow-up since then at one of the largest Hungarian autoimmune centers. We compared the clinical characteristics, therapy, survival, causes of death, and prognostic factors of LN and non-LN lupus patients. Furthermore, in order to shed light on the achievements of the last decades, we compared a wide spectrum of clinical data as well as treatment modalities of LN patients diagnosed before and since 2005, and determined any changes in disease course and outcomes.

In the second part of our work, data on the white population of Central Europe is limited and Hungarian data on the clinical characteristics of lupus nephritis are not available from the past 10 years. It is not known whether there is a difference in the course of lupus nephritis between the histological classes. It is also unknown which further factors may affect the progression of CKD. Furthermore, the differences in clinical course and laboratory parameters between patients with complete renal response (CR), partial renal response (PR) and non-responder status (NR) are not fully known. In view of all this, we found it worthwhile to investigate the parameters mentioned above and to assess prognostic factors for the unfavourable renal outcome.

2. Objectives

Several research groups consider lupus nephritis (LN) a negative prognostic factor. At the same time, diagnostic and therapeutic options have also evolved over the past decades. Therefore, our goal was to examine, at the Department of Internal Medicine, Clinical Immunology Division of the Faculty of Medicine, University of Debrecen:

1. The prevalence of lupus nephritis (LN).
2. To compare the clinical features, treatments, survival rates, causes of death, and to identify prognostic factors between patients with and without LN.
3. To analyze a total of 30 years by comparing two 15-year periods, observing how the clinical presentation and management of lupus nephritis have changed in our center.

In the second part of our work, we focused on data from patients with lupus nephritis. No recent Hungarian data are available regarding the clinical characteristics of lupus nephritis over the past 10 years.

1. We examined whether there are differences in the course of lupus nephritis among different histological types.
2. We sought to identify prognostic factors that may influence the progression of chronic kidney disease and the development of end-stage renal disease.
3. We investigated the clinical course and laboratory parameters in patients with complete, partial, or no response to therapy.

3. Materials and methods

3.1. First study population

In our retrospective study, we assessed the data of 384 Hungarian patients who were diagnosed with SLE between 1990 and 2020, and followed up regularly at the Division of Clinical Immunology (Faculty of Medicine, University of Debrecen), which is one of the largest tertiary referral centers in Hungary for systemic autoimmune diseases. All patients enrolled in our study fulfilled the EULAR/ACR 2019 classification criteria for lupus. SLE patients with secondary antiphospholipid syndrome (APS) fulfilled the 2006 Sydney criteria; moreover, all of them met the criteria of the 2023 ACR/EULAR APS classification criteria, as well.

3.2. Second Study population

In our retrospective cohort study, we gathered the data of 384 Hungarian SLE patients, all of whom were white Europeans from the Central European region. They were diagnosed with SLE between 1990 and 2022, and have received therapy and regular follow-up care at the Division of Clinical Immunology, Institute of Internal Medicine, Faculty of Medicine, University of Debrecen. Patients diagnosed with SLE before 2012 were revised according to the SLICC criteria for SLE; additionally, all SLE patients also fulfilled the EULAR/ACR 2019 classification criteria for lupus. SLE patients with secondary antiphospholipid syndrome (APS) fulfilled the 2006 Sydney criteria; of note, all of them met the criteria of the 2023 APS ACR/EULAR classification criteria, as well. Lupus nephritis was diagnosed in 127 of them, therefore only these patients were included in the further examinations of the present study. The clinical and laboratory data of patients were extracted from medical documentations and records for statistical analyses.

3.3. Clinical and laboratory evaluation

All patients were routinely followed up throughout the studied period, and their medical records contained detailed information on medical history, treatments as well as clinical symptoms, physical conditions and laboratory and other findings of each visit. The following demographic and clinical data were analysed: sex, age, age at diagnosis, disease duration, histological class of lupus nephritis, clinical symptoms and laboratory results, immunoserological abnormalities, and applied treatments during the disease course. The renal biopsies were performed at the Department of Nephrology (Institute of Internal Medicine, Faculty of Medicine, University of Debrecen), and the kidney biopsy samples were evaluated at the Department of Pathology (Faculty of Medicine, University of Debrecen). The calculation of activity and chronicity indices was introduced for patients diagnosed after 2005, so 83 patients (20 with CKD stage 3-5 and 63 with CKD stage 1-2) had data about LN Activity and Chronicity Index. We used the current WHO classification system, ISN/RPS2003, ISN/RPS2018 for histological description. We defined CKD stages according the KDIGO guidelines. We combined CKD stage 1 and stage 2 into a single category on the basis of $eGFR \geq 60$ ml/min per 1.73 m^2 . CKD stage 3 was described as $eGFR 30$ to <60 ml/min per 1.73 m^2 , CKD stage 4 $eGFR 15$ to <30 ml/min per 1.73 m^2 for at least 3 consecutive months. CKD stage 5 (ESRD) was defined by $eGFR \leq 15$ ml/min per 1.73 m^2 for at least 3 consecutive months or ongoing dialysis. In the case of a CR, the degree of proteinuria decreased below 0.5 g in 6 months, and the creatinine level returned to the initial value. In case of PR, the degree of proteinuria was halved in 6 months, serum creatinine level stabilized or improved, but did not return to baseline. Chronic organ damage in SLE was determined using the SLICC damage index. Immune serological parameters were determined from serum samples. The presence of antinuclear antibodies (ANA) was detected by an indirect immunofluorescence method on the HEp2 cell line. Enzyme linked immunosorbent assay (ELISA) was used for the detection of the following antibodies: anti-dsDNA (Orgentec, Mainz, Germany), anti-SS-A, anti-SS-B, anti-RNP, anti-Sm (Hycor, Biomedical, Garden Grove, California, USA), as well as antiphospholipid antibodies including anti-cardiolipin (aCL) IgG/IgA/IgM and anti- $\beta 2$ GPI IgG/IgA/IgM antibodies (Orgentec, Mainz, Germany). All laboratory tests were performed under standardized conditions, according to the manufacturer's instructions, at the Department of Laboratory Medicine, Faculty of Medicine, University of Debrecen.

3.4. Statistical analysis:

First study: Statistical analysis was performed using SPSS Statistics for Windows, Version 28.0 (IBM Corporation, Armonk, NY, USA) and GraphPad Prism version 9.5 for Windows (GraphPad Software, San Diego, California USA). Values are expressed as mean and standard deviation (SD) or median with interquartile range (IQR) for continuous variables, and frequency with percentage for categorical variables. Continuous variables were compared with parametric Student's t-test for two samples or with the nonparametric Mann-Whitney U test. Categorical variables were compared with Pearson's chi-squared test or Fisher's exact test. Kaplan-Meier analysis was used to estimate survival from the diagnosis and log-rank test was used to compare the survival curves. Significant variables on log-rank tests were further evaluated with univariate Cox proportional hazards regression models with estimation of hazard ratios (HR). Receiver operating characteristic (ROC) curve analysis was used to determine the optimal cut-off value for SDI as a prognostic factor for mortality. All statistical test were two-sided, differences were considered statistically significant at <0.05 level and reported using p-values and/or 95% confidence intervals (95% CI).

Second study: Statistical analysis was performed using SPSS Statistics for Windows, Version 28.0 (IBM Corporation, Armonk, NY, USA) and GraphPad Prism version 9.5 for Windows (GraphPad Software, San Diego, California USA). Shapiro-Wilk test of normality was conducted to determine whether continuous data is normally distributed. Values are expressed as median with interquartile range (IQR) for continuous variables, and frequency with percentage for categorical variables. Continuous variables were compared with nonparametric Mann-Whitney U test. Categorical variables were compared with Pearson's chi-squared test or Fisher's exact test. Univariate and multivariate binary logistic regression analysis were performed to identify predictors for the progression of CKD. First we performed univariate analysis using variables with $p<0.05$ in Mann-Whitney U test or Pearson's chi-squared test and Fisher's exact to avoid over-fitting, only variables with $p<0.05$ in univariate binary logistic regression analysis were included in multivariate analysis. Receiver operating characteristic (ROC) curve analysis was used to determine the optimal cut-off value for AI and CI as prognostic factors for the pregression of CKD into stage 3-5 and the development of ESRD. All statistical test were two-sided, differences were considered statistically significant at <0.05 level and reported using p-values and/or 95% confidence intervals (95% CI).

4. Results

4.1. First study

4.1.1. Demographic characteristics

The population of our retrospective study consisted of 384 Hungarian SLE patients, of whom 339 (88.3%) were women and 45 (11.7%) were men. Their mean age at the time of data collection was 50.8 ± 13.4 years, their age at the time of SLE diagnosis was 33.3 ± 11.9 years, and the average duration of disease was 17.5 ± 8.0 years. Among the examined patients, 127 (33.1%) had lupus nephritis, while 257 patients (69.9%) did not have LN. Table 1 shows the demographic data of patients. Patients with LN were significantly younger both at the time of the SLE diagnosis and the data collection. The women : men ratio was the same in both groups; of note, LN was not more prevalent in male patients.

4.1.2. Clinical characteristics and laboratory findings of LN and non-LN patients

We compared the clinical characteristics and laboratory findings of LN and non-LN patients. Among the clinical symptoms and associated diseases, subacute cutaneous lupus erythematosus (SCLE), discoid lupus erythematosus (DLE), secondary APS, Sjögren's syndrome and rheumatoid arthritis were significantly more common in the non-LN group. Regarding laboratory results, LN patients were affected by anemia and anti-RNP positivity significantly more frequently. We did not find any other significant difference in clinical or laboratory parameters between the two groups.

4.1.3. Treatment modalities for LN and non-LN patients

When comparing the treatment of SLE, we found that corticosteroids, azathioprine, cyclophosphamide, mycophenolate mofetil and rituximab were used significantly more frequently in LN patients. On the contrary, the use of chloroquine and methotrexate was significantly more common in non-LN patients.

4.1.4. Survival rates and causes of death

The 5-, 10-, 15-, 20-, 25- and 30-year survival rates in LN group were 97.6%, 94.9%, 87.3%, 84.3%, 78.9% and 75.3%, respectively. The 5-, 10-, 15-, 20-, 25- and 30-year survival rates in non-LN group were 99.6%, 96.6%, 92.7%, 87.8%, 83.4% and 83.4%, respectively. The mean survival time was 28.2 years (95% CI 26.6-29.8) in LN group, and 29.3 years (95% CI 28.4-30.3) in non-LN group. We did not find a significant difference in survival between the two groups ($p=0.232$).

Forty-five deaths (11.7%) occurred in the entire patient group. Thirteen (28.9%) patients died of cardiovascular events, and 12 (26.7%) patients due to some kind of infection. We lost 11

(24.4%) patients due to malignancy, 6 (13.3%) patients due to a severe fulminant sepsis, while 3 (6.7%) patients died of other causes. There were 18 deaths in the LN group, mainly due to cardiovascular events (n=5, 27.8%) and sepsis (n=5, 27.8%), the latter was significantly more common in this group compared to the non-LN group. We observed 27 cases of death in the non-LN group, the most common causes were cardiovascular events (n=8, 29.6%), and malignancies (n=8, 29.6%).

We looked for factors that could determine the survival rates of patients. In the LN group, there was no significant difference between the sexes in survival ($p=0.051$). There were 14/114 (12.3%) deaths among women and 4/13 (30.8%) among men. In the Non-LN group, a significant difference was observed between the survival of women and men ($p<0.001$). There were 17/225 (7.6%) deaths among women and 10/32 (30.8%) among men. Based on the result from the univariate Cox regression, the prognosis of men in the non-LN group is worse, compared to women; the hazard ratio (HR) is 5.43 (95% CI 2.47-11.92, $p<0.001$).

Regarding the effect of achieving lupus low disease activity state (LLDAS) on survival, we found that patients with LLDAS had significantly better survival in LN group ($p=0.038$).

Death in patients who achieved LLDAS was 1/23 (4.3%), while in patients who did not achieve LLDAS, it was 17/82 (20.7%). In the non-LN group, LLDAS did not significantly affect survival ($p=0.131$). Death in patients who achieved LLDAS was 3/43 (7.0%), while in patients who did not achieve LLDAS, it was 24/160 (15.0%).

The survival of patients in remission was significantly better in both patient groups (LN: $p=0.014$; non-LN: $p=0.002$). In the case of patients who did not achieve remission, there were 17/82 (20.7%) deaths in the LN group, while 24/160 (15.0%) in the non-LN group. In patients who achieved clinical or complete remission, there were no deaths in either group.

We also examined the SDI's impact on survival. Based on the ROC analysis, the SDI's optimal cut-off value was 2 points in both groups; the area under the curve (AUC) in the LN group was 0.655 ($p=0.036$, 95% CI 0.51-0.80, cut-off value: 2 points), while 0.807 in the non-LN group ($p<0.001$, 95% CI 0.74-0.88, cut-off value: 2 points).

SDI did not affect the survival in the LN group ($p=0.347$). On the other hand, in the non-LN group, SDI had a significant effect on survival ($p=0.004$). Based on the result from univariate Cox regression, the survival of those with more than 2 points in the non-LN group was significantly worse compared to those with fewer points (HR=2.93 [95% CI 1.37-6.28]; $p=0.006$).

4.1.5. Differences between patients LN patients diagnosed before 2005 or since 2005

In the second part of the research, we were interested in whether the clinical characteristics of patients with LN had changed. Therefore we divided LN patients into two groups and compared the clinical characteristics as well as treatment modalities of LN patients diagnosed before and since 2005. The follow-up data of patients diagnosed before 2005 were then only taken into account until 2005, so that the comparison between the two patient groups would be proportional.

Based on our results, the prevalence of many clinical manifestations of SLE has decreased in the last 15 years compared to before. Raynaud's phenomenon, DLE, pleuritis, pericarditis, central nervous system and psychiatric manifestations as well as stroke became significantly less prevalent. The frequencies of chronic kidney disease and end-stage renal disease also showed a decreasing trend, although the differences were not significant. The SDI of patients improved significantly in the past 15 years. There have also been changes regarding the prescribed medicines. The use of cyclophosphamide significantly decreased, while the use of mycophenolate mofetil, chloroquine and rituximab significantly increased. At the same time, the cumulative corticosteroid dose was significantly reduced.

4.2. Second study

4.2.1. Main analyses

The population of our retrospective study consisted of 127 LN patients, 114 (89.8%) women and 13 (10.2%) men. Their age at the time of SLE diagnosis was 28.4 ± 9.9 years, and they were followed up regularly at our Autoimmune Center for 16.9 ± 8.1 years. All patients included in the study underwent a renal biopsy. The most common histological type was class IV LN, followed by class V, then class III. Mixed histological types were found in 11 patients; while for two patients it was not possible to identify the histological type (they were diagnosed before 2000, the biopsy was taken in another centrum and the reclassification was not possible). We did not detect class VI LN in our patient group. CKD stage 3-5 developed in 35 patients; among them, 12 patients had CKD stage 5 (ESRD). At the time of the data collection, 71.6% of patients were in CR, 15% were in PR, while 13.4% were non-responders to the therapy.

4.2.2. Comparison of patients based on the stage of CKD

We compared the demographics, clinical characteristics, laboratory parameters, therapies, outcome and mortality of CKD stage 3-5 patients with the ones with CKD stage 1-2, as well as CKD stage 5 (ESRD) patients with the ones with CKD stage 1-4 (non-ESRD).

CKD stage 3-5 patients were significantly older at the time of data collection than CKD stage 1-2 patients, while there was no difference between the ages of ESRD and non-ESRD patients. We found no differences between neither CKD stage 3-5 and CKD stage 1-2, nor ESRD and

non-ESRD groups in SLE clinical symptoms, autoantibodies, and cardiovascular complications. The association with Sjögren's syndrome was significantly more common in CKD stage 3-5 group, compared to CKD stage 1-2 patients. Coombs positivity was significantly more common in CKD stage 3-5 patients, while anti-DNA positivity was significantly less common. Anti-RNP and anti-SS-B autoantibody positivity was detected significantly more frequently in ESRD patients, compared to non-ESRD patients. The occurrence of class IV LN was significantly more frequent in CKD stage 3-5 group; on the other hand, class III LN did not occur in patients with CKD stage 3-5 at all. The chronicity index as well as SDI score was significantly higher in both CKD and ESRD groups, compared to CKD stage 1-2 and non-ESRD patients. The administration of immunosuppressive therapy did not differ between neither CKD stage 3-5 and CKD stage 1-2, nor ESRD and non-ESRD groups. The cumulative steroid dose was significantly higher in CKD stage 3-5 group compared to patients with CKD stage 1-2. A CR developed significantly less frequently in CKD stage 3-5 as well as in ESRD group. None of the ESRD patients reached CR. Consequently, the ratio of non-responder patients was significantly higher in both CKD and ESRD groups. The total mortality rate was significantly higher in patients with CKD stage 3-5, than in patients with CKD stage 1-2; however, there was no difference regarding the causes of death.

4.2.3. Prognostic factors for the progression of CKD and the development of ESRD

Univariate and multivariate logistic regression analyses revealed that age above 42 years, class IV lupus nephritis, Coombs positivity and a higher chronicity index are important prognostic factors for the development of CKD stage 3-5. Univariate logistic regression analysis revealed that anti-RNP and anti-SS-B positivity as well as high chronicity index are important prognostic factors for the development of ESRD. Of note, multivariate logistic regression analysis confirmed only the high chronicity index among these. We also evaluated the possible associations between activity and chronicity index scores and the development of CKD stage 3-5 and ESRD. Activity score did not show any correlation with the development of CKD stage 3-5 or ESRD. However, the chronicity index score above 3.5 points showed a significant correlation with the development of both CKD stage 3-5 and ESRD.

4.2.3. Differences between class IV LN and non-class IV LN patients

Given that class IV LN was the most common histological type and a prognostic factor of the development of CKD stage 3-5, we compared it with other non-class IV LN. Class IV LN patients were younger than the others at the time of data collection; however, their age at diagnosis did not differ. Regarding the clinical features, deep vein thrombosis as well as rheumatoid arthritis were significantly less common in class IV LN patients. During the

assessment of kidney pathology we found that both the activity and chronicity indexes were significantly higher, and the development of CKD stage 3-5 was more prevalent in the class IV lupus nephritis patients. We did not find any difference in immunoserological results. Azathioprine was significantly more frequently used as maintenance treatment in class IV LN group, while cyclosporine A was used significantly less often. Total mortality was not more frequent in class IV lupus nephritis group, but among the causes of death, tumour mortality was significantly less frequent.

4.2.4. Differences between patients with complete (CR), partial renal response (PR) and non-responders

The age at data collection was significantly higher, and the duration of disease was significantly longer in the patients with CR than the patients with PR. Regarding autoantibodies, no differences were found in the three groups. The occurrence of CKD stage 3-5 and ESRD was significantly more frequent among non-responders compared to patients with CR, and their chronicity index was also significantly higher. Steroid use was significantly more frequent among patients with PR compared to patients with CR. MMF induction and maintenance treatments were used significantly more often in patients with PR. MMF induction therapy was significantly more common among non-responders compared to patients with CR. Both tacrolimus and belimumab were used significantly more often in patients with PR than with CR. Patients with CR achieved lupus low disease activity state (LLDAS) more common than patients with PR. The SDI score was significantly higher among non-responders compared to those with CR.

5. Discussion

Lupus nephritis is a clinical manifestation that fundamentally influences the prognosis of SLE. LN can develop at any time during the course of the disease; nevertheless, its development most often occurs in the first 3-5 years of SLE, and sometimes LN can be one of the first symptoms of lupus. The prevalence and severity of LN shows geographic and ethnic variety. It may vary within the white population, as the Greek researchers found a LN prevalence of 20.3% among 555 SLE patients, while the Spanish RELESSER registry with more than 4000 SLE patients' data showed a LN prevalence similar to our results, in which almost one third of SLE patients were affected. Studies from other countries of the world showed an even higher LN prevalence. An Iranian study with a large number of SLE patients revealed a prevalence of 68.1%, Chinese authors reported 49-58% prevalence, while a Columbian research found a 51% prevalence. In

most studies, the group of lupus patients with LN was significantly younger both at the time of diagnosis and at the time of data collection. We compared the clinical characteristics SLE patients between LN and non-LN patient groups. We found that the prevalence of SCLE, DLE is higher in the non-LN group, in which other systemic autoimmune diseases are also more common, such as secondary APS, Sjögren's syndrome and rheumatoid arthritis. Among our patients with lupus nephritis, only anemia was observed to be more common; hematological manifestations also occurred more frequently in Spanish LN patients. The Iranian workgroup found photosensitivity, malar rash and central nervous system symptoms to be more common among LN patients, while polyarthritis and autoimmune hemolytic anemia occurred mostly among non-lupus nephritis patients. Based on data from the Spanish registry, serositis, skin symptoms, neurological manifestations, pulmonary involvement and hematological diseases associate more commonly with LN. We could not confirm these findings, and contrary to the Spanish data, the association of APS was more common in non-LN Hungarian patients. Colombian authors reported a higher prevalence of hypertension and pleurisy in LN patients, furthermore; they also found anti-DNA positivity to be more common. It is known that the presence of several autoantibodies can be associated with the occurrence of lupus nephritis. The association of anti-Sm, anti-nucleosome or anti-C1q antibodies with lupus nephritis is well known. We found anti-RNP to be significantly more common among patients with lupus nephritis; on the other hand, based on data from the RELESSER registry, anti-Sm is more common, while the Iranian working group found ANA positivity to be more common in their patients with LN. We found no difference in the prevalence of ANA, anti-dsDNA or anti-Sm antibodies. Since the measurement of anti-nucleosome and anti-C1q antibodies was not performed in all of our patients, we did not evaluate their presence. There is a working group that has associated anti-SSA with worse renal outcomes, The literature is conflicting regarding the presence of anti-SS-A. Some authors associated anti-SS-A with a worse renal outcome; on the contrary, the Colombian authors reported a protective role of anti-SS-A. We found no difference in the presence of anti-SS-A between our two patient groups.

As expected, LN patients were treated with cyclophosphamide, MMF or rituximab more often, while antimalarial drugs were given less often. This can be explained by the fact that previously it was not the practice for all patients to receive antimalarial treatment. At the same time, when we examined the therapeutic changes of the last 15 years, the use of chloroquine in LN patients increased significantly. Similar changes occurred with other immunosuppressants, MMF is more frequently used as both induction and maintenance therapy. It is known that the effectiveness of cyclophosphamide and MMF is the same, but the side effect profile of MMF

is more favorable, thus we prefer it in the case of young patients of fertile age, therefore the use of MMF has increased, while the use of cyclophosphamide has decreased. We observed another favorable therapeutic change, the cumulative steroid dose has significantly reduced, which has a significant role in the reduction of chronic organ damage.

According to our results, the clinical picture of lupus nephritis has also changed. Despite the fact that the investigated clinical manifestations of SLE were not more common among LN patients compared to non-LN patients, there were changes in the prevalence of several organ abnormalities over the last 15 years. The prevalence of serositis and neuropsychiatric manifestations decreased significantly, as did the SDI. Recently, Mok and his colleagues compared data from two 10-year periods. They found that patients diagnosed in the second 10-year period had anti-ENA antibodies more frequently, which is probably attributable to the developing laboratory methodology; we did not find similar difference. They also showed, like us, that the SDI score also decreased significantly, which has an important impact on long-term survival. Based on our results, the SDI had an effect on the survival of non-LN patients, a damage score above 2 points significantly worsened their survival, while it did not affect the survival of LN patients. An Italian working group examined the clinical and histological changes in LN and the disease outcome in nearly 500 patients over a period of 50 years. They concluded that the severity of lupus nephritis has decreased in the past years, and the outcome of disease has also improved, which findings are confirmed by our results.

Examining survival, we found no significant difference in the short- and long-term survival of LN and non-LN patients, although after 15 years the indicators of LN patients are worse, the difference is not statistically significant. Male sex did not significantly worsen survival among LN patients, while it proved to be a negative prognostic factor in non-LN patients. When examining the survival of SLE patients, Doria et al. identified male sex, the presence of lupus anticoagulant, and severe disease as negative prognostic factors, and found that glomerulonephritis also worsened survival rates. On the contrary, Lou et al. found no difference in the survival of either LN or the overall SLE population in terms of sex, and reported that younger age and non-Caucasian race worsened the survival of patients. We also examined how achieving low disease activity and remission affects patients' survival rates. It became clear that the achievement of remission significantly improves survival in both the LN and non-LN groups, while low disease activity is protective only in lupus nephritis group. Based on the results of a prospective multicenter study, LLDAS significantly improved patient survival and remission did not add to it. Our results showed that low disease activity is sufficient in patients with lupus nephritis, but in the non-LN group, the therapeutic goal should be to achieve

remission. Achieving LLDAS and remission reduces the development of chronic damage and reduces SDI, thus contributing to improved survival. The leading causes of death for our patients correspond to international data, according to which cardiovascular, infections and tumors are the main causes of death.

Cardiovascular diseases are the leading cause of death in both lupus nephritis and non-LN patients. The occurrence of severe sepsis as death cause was significantly more frequent in LN group, which is presumably explained by the more potent immunosuppressant treatment.

In the second part of our work the patients with lupus nephritis were investigated. The most common histological type is class IV LN, and we found it in nearly 60% of our patients. Similar frequency was reported by Vajgel et al.; although, in a non-white population. CKD stage 3-5 developed in 27.6% of our patients. However, when comparing our observations with the results of other working groups, it is particularly important to highlight the following fact. LN implies persistent alterations to kidney structures and functions, therefore all LN patients develops CKD, and the CKD stage of these patients determines their health outcomes. In the publications published so far in the literature, the definition used by each working group for the diagnosis of CKD in their LN patients corresponded to our definition of CKD stages 3-5 (defined by eGFR <60 ml/min per 1.73 m² for at least 3 consecutive months). Consequently, their definition for non-CKD LN patients corresponds to the definition of LN patients with CDK stage 1-2 in our study. An Egyptian working group observed a CKD rate of 32.7% in a 5-year study of more than 900 patients with lupus nephritis; however, these data come from a non-white population. The frequency of ESRD in our patient population was 9.4%, which is similar to the data of RELESSER register (10.35%). Platinga et al. found the 5-year cumulative incidence of ESRD to be 2.5% in white and 6.4% in black individuals. Similar results were obtained by Hanly et al., for all patients with LN, the 5-year cumulative incidence of ESRD was 3.3%, while the 10-year cumulative incidence was 4.3%. When we evaluated the demographic, clinical, laboratory data and renal outcomes of CKD stage 3-5 patients, we found that they were older, and the association of Sjögren's syndrome was more common, compared to the patients with CKD stage 1-2. Park et al. found CKD patients to be similarly older in a Korean population. Similar to these results, the clinical manifestations of SLE did not differ between our patients of CKD stage 3-5 and CKD stage 1-2 groups. At the same time, in the Korean cohort, hypertension, elevated serum creatinine level, reduced eGFR and nephrotic proteinuria were more common in CKD group at the time of LN diagnosis. We could not examine these parameters, as the initial laboratory parameters were not available for all patients due to the long follow-up period. We found that class IV lupus nephritis and a higher chronicity index are significantly more

common in patients with CKD stage 3-5. It is similar to the results of other studies; however, we found no correlation with the activity scores. Direct Coombs positivity was also more common in CKD stage 3-5 group, but anti-DNA positivity was significantly less frequent, which contradicts the literature data. Based on a Spanish register data, ESRD showed a significant association with anti-DNA positivity; however, it was also described that there is a significant correlation between hemolytic anemia and ESRD, and they did not comment on isolated Coombs positivity. Regarding the therapy, we found no difference in CKD stage 3-5 and CKD stage 1-2 groups, except for the higher cumulative steroid dose. The reason for this may be that CKD patients had a more severe form of LN and a higher dose of steroid was needed. Not surprisingly, among patients with CKD stage 3-5, there were fewer patients in CR and more non-responders. We observed higher SDI scores and mortality rate in CKD stage 3-5 group, but there was no difference in causes of death. Based on our results, age above 42 years, class IV LN, Coombs positivity and higher chronicity index proved to be important prognostic factors for the development of CKD stage 3-5. Formerly, several working groups identified hypertension, elevated serum creatinine levels, and lack of remission as prognostic factors. A higher chronicity index, on the other hand, is described by numerous working groups as a prognostic factor for CKD and ESRD. We found the score of 3.5 points to be a value above which the development of CKD stage 3-5 and ESRD must be more anticipated.

We compared the data of ESRD and non-ESRD patients as well. We found no differences in the clinical symptoms of SLE, but anti-RNP and anti-SS-B positivity were significantly more frequent in ESRD group. Several working groups have described anti-SS-A as an independent predictor of ESRD, without mentioning anti-SS-B. Anti-RNP and anti-SS-B positivity as well as a higher chronicity index were found to be prognostic factors for the development of ESRD; however, multivariate analysis confirmed the prognostic value of higher chronicity index only. Other study groups found hypocomplementemia, class III, IV, VI lupus nephritis, hypertension, older age, male sex and black race to be the main prognostic factors for ESRD. We did not find class III LN to be more frequent; moreover, this class did not even occur in neither the CKD stage 3-5, nor the ESRD group.

Given that class IV LN was the most common histological type, and CKD stage 3-5 and ESRD did not occur in class III LN patient group, as next step, we compared class IV and non-class IV LN patients. Duran et al. examined the clinical characteristics and disease outcome of proliferative and non-proliferative LN patients. They found no difference in SLE clinical symptoms between the two groups, just as we found no difference between the class IV and non-class IV LN patients. They found significant differences though in several parameters

indicating kidney activity, such as serum creatinine level, eGFR, proteinuria, hypocomplementemia, active urine sedimentation and renal SLE DAI. We did not examine these parameters in our study. We found a significantly higher activity and chronicity index in the class IV LN patients. A Columbian work group obtained similar results when comparing patients with proliferative and non-proliferative LN. In terms of therapy, we found a difference only in the administration of cyclosporine A. We used significantly less often in class IV LN, similarly to Duran et al. We found no difference in mortality between patients with class IV and non-class IV LN; however, the tumour mortality was significantly lower among class IV LN patients.

Finally, we also compared patients with CR or PR and non-responders. Patients with CR were older and had a longer disease duration compared to patients with PR. The damage index, and the prevalence of CKD stage 3-5 and ESRD were significantly higher among the non-responders. We found no differences between the individual groups in terms of clinical symptoms and laboratory abnormalities, but there was a difference in their treatment. Patients with PR received newer drugs, such as MMF, tacrolimus and belimumab significantly more frequently. In the therapeutic recommendation of lupus nephritis, these drugs have been introduced in recent years, and the latest KDIGO guidelines and ACR/EULAR recommendations also include them. Presumably, the newer and registered drugs will be used more and more widely, not only in patients with PR, but also in the entire lupus nephritis patient population. Among immunosuppressive treatments, steroid therapy was also more common in patients with PR. Patients with CR currently took steroids less often, which is also in line with the current therapeutical guidelines and our therapeutic efforts. Nevertheless, even more attention should be paid to the use of antimalarial drugs, because half of our patients received only antimalarial drugs, even though it is well-known that HCQ has a beneficial effect on the outcome of the disease in many respects. Park et al. also reported that the use of hydroxychloroquine before the onset of lupus nephritis protects patients from developing CKD. When examining the disease outcome, we found that mortality did not differ in each group, but LLDA was significantly more prevalent with CR group, which may improve the morbidity and mortality indicators of patients in the long term. At the same time, we found a higher damage index among non-responders, which may have a negative effect on the long-term outcome.

6. Summary

Lupus nephritis (LN) is one of the most severe organ manifestations of systemic lupus erythematosus (SLE). Chronic kidney disease (CKD) and its progression into end-stage renal disease (ESRD) are serious complications in LN, and the main cause of death in SLE. We aimed to investigate the prognostic factors for the progression of CKD and the development of ESRD in SLE patients.

In the first part of our cohort study, we assessed the clinical and laboratory data of 384 SLE patients, out of whom 127 patients were diagnosed with LN. We also compared LN patients diagnosed before and since 2005. In the second part of our retrospective cohort study, we assessed the clinical and laboratory data of 127 patients who were diagnosed with LN between 1990 and 2022, and received regular follow-up care at our autoimmune center. We compared class IV (diffuse) LN patients with non-class IV LN patients, and assessed the differences in clinical and laboratory data of patients subdivided into complete, partial and non-responders to therapy

In the first part of our work, based on our observation, discoid LE, subacute cutaneous LE, antiphospholipid syndrome, Sjögren's syndrome and rheumatoid arthritis were more common in non-LN patients, while anemia and anti-RNP positivity were more frequent in LN patients. The development of LN did not affect survival rates significantly; male sex was a negative prognostic parameter, while achieving remission was a positive prognostic factor in both groups. Death caused by sepsis was more prevalent in LN group. Serositis and neurological manifestations occurred less frequently in LN patients diagnosed after 2005. The use of mycophenolate mofetil became more common, and the cumulative steroid dose decreased. The SLICC Damage Index score also decreased. Our study demonstrated that the disease course has changed in recent years, and the main therapeutic goal in both SLE and lupus nephritis should be to achieve remission, because this significantly improves long-term prognosis and patient survival. Our study demonstrated that the disease course has changed in recent years, and the main therapeutic goal in both SLE and lupus nephritis should be to achieve remission, because this significantly improves long-term prognosis and patient survival.

In the second part of our work, the prevalence of class IV LN is significantly higher in patients with CKD stage 3-5. Age above 42, class IV LN, Coombs positivity and high chronicity index are prognostic factors for the development of CKD stage 3-5. On the other hand, anti-RNP and anti-SS-B antibody positivity and a high chronicity index are prognostic factors for the development of ESRD. The chronicity index as well as SLICC/ACR Damage Index (SDI) score

were significantly higher in non-responders compared to patients with complete remission. Based on our results, the progression of CKD into stage 3-5 or the development of ESRD should be expected at a chronicity index above 3.5 points. An early diagnosis as well as aggressive and adequate treatment on time are fundamental to prevent unfavourable outcome of LN.

7. Summary of new observations

1. The occurrence of DLE, SCLE, Sjögren's syndrome, rheumatoid arthritis, and antiphospholipid syndrome is more common among Hungarian SLE patients without kidney involvement.
2. Anti-RNP positivity is more frequent in Hungarian lupus nephritis patients.
3. Achieving low disease activity or remission significantly improves the survival of lupus nephritis patients.
4. A damage index above 2 significantly worsens the survival of non-lupus nephritis patients, but such an effect is not observed in SLE patients with lupus nephritis.
5. Age over 42 years and Coombs positivity are risk factors for the development of CKD stages 3-5.
6. The development of CKD stages 3-5 and ESRD is significantly more likely in patients with a damage index above 3.5 compared to those below.
7. Patients showing complete renal response have a lower proportion of steroid use compared to non-responders.
8. Sepsis contributes more significantly to mortality in lupus nephritis patients than in non-lupus nephritis patients.

8. Acknowledgments

I would like to express my gratitude to my supervisor, *Dr. Tünde Tarr* who supported and guided my scientific work, assisted in planning my PhD research, evaluating the results, and preparing the dissertation. Her professional and personal advice has been helpful in everyday life as well, for which I am grateful.

I also wish to thank † *Prof. Dr. Margit Zeher*, previous head of the Department of Clinical Immunology, for enabling my participation in scientific research. I thank *Professor József Balla*, head of the Department of Internal Medicine and Nephrology, for allowing us to contribute professionally and scientifically for the benefit of kidney patients.

Many thanks to *Dr. Csilla Markóth* for performing kidney biopsy examinations and to *Dr. László Bidiga* for his work in nephropathological histological evaluation.

I am grateful to *Dr. Gábor Papp* for his assistance during my scientific work, and to *Bernadett Bói* for help with statistical analyses.

Thanks to *Judit Baranyi* for technical support.

I would like to express my gratitude to my co-authors and *colleagues* who helped and supported my scientific work through their collaboration.

I am also deeply thankful to my *friends* and, most of all, *my family*, who always support me with patience.

9. List of publications



Registry number: DEENK/75/2025.PL
Subject: PhD Publication List

Candidate: Bianka Perge

Doctoral School: Gyula Petrányi Doctoral School of Allergy and Clinical Immunology

List of publications related to the dissertation

1. Perge, B., Papp, G., Bói, B., Markóth, C., Bidiga, L., Farmasi, N., Balla, J., Tarr, T.: Prognostic Factors of the Progression of Chronic Kidney Disease and the Development of End-Stage Renal Disease in Patients with Lupus Nephritis: a Retrospective Cohort Study. *J. Clin. Med.* 14 (3), 1-18, 2025.
DOI: <http://dx.doi.org/10.3390/jcm14030665>
IF: 3 (2023)
2. Perge, B., Papp, G., Bói, B., Nagy, N., Gáspár-Kiss, E., Tarr, T.: Clinical Features and Survival Analysis of Lupus Nephritis among Patients with Systemic Lupus Erythematosus: a Three-Decade-Long Retrospective Cohort Study. *Biomedicines.* 12 (9), 1-17, 2024.
DOI: <http://dx.doi.org/10.3390/biomedicines12092117>
IF: 3.9 (2023)

List of other publications

3. Nagy, N., Bói, B., Papp, G., Fiák, E., Gáspár-Kiss, E., Perge, B., Farmasi, N., Tarr, T.: Antiphospholipid Antibodies Are Major Risk Factors for Non-Thrombotic Cardiac Complications in Systemic Lupus Erythematosus. *Biomedicines.* 12 (3), 1-15, 2024.
DOI: <http://dx.doi.org/10.3390/biomedicines12030530>
IF: 3.9 (2023)
4. Diószegi, Á., Lőrincz, H., Kaáli, E., Soltész, P., Perge, B., Varga, É., Harangi, M., Tarr, T.: Role of Altered Metabolism of Triglyceride-Rich Lipoprotein Particles in the Development of Vascular Dysfunction in Systemic Lupus Erythematosus. *Biomolecules.* 13 (3), 1-13, 2023.
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IF: 4.8





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Med. Tribune. 18 (2), 14-15, 2020.
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Joint Bone Spine. 83 (6), 675-679, 2016.
DOI: <http://dx.doi.org/10.1016/j.jbspin.2015.10.017>
IF: 3.329

Total IF of journals (all publications): 18,929

Total IF of journals (publications related to the dissertation): 6,9

The Candidate's publication data submitted to the Tudóstér have been validated by DEENK on the basis of the Journal Citation Report (Impact Factor) database.

27 February, 2025

