LUPUS NEPHRITIS - CLINICAL AND THERAPEUTICAL ASPECTS

Viorica Crisan

INTRODUCTION

Systemic lupus erythematosus (SLE) is an inflammatory chronic disease with multisystemic involvement, with different clinical and immunological manifestations, characterized by the presence of antinuclear antibodies.\(^1,2\)

Renal involvement in SLE occurs in 40-75% patients, most frequently in a period of 5 years after the onset of the disease. It is one of the strongest predictors of a poor outcome.

Renal glomeruli are the main affected structures by the immune complexes deposits.\(^2\)

The clinical manifestations of the renal involvement occur in 50% patients and depend on the histological type of LN. The persistent proteinuria is the most frequent sign of the LN and occurs in 70% of patients. Hematuria occurs in 40% of patients and azotemia in 30%. A proteinuria under 1 g/day means mesangial glomerulonephritis (GN), proteinuria between 1-3 g/day means focal or diffuse GN, proteinuria > 3 g/day means GN membranous or proliferative diffuse.\(^3\)

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The treatment is individual and depends on the clinical form, histological type, functional status, disease activity, age, sex.

The general measures consist of low salt intake (5 g NaCl/24 hours), lipid and protein diet (0.8 g/Kg/24 hours). Hypokalemia is corrected with KCl 1-2 g/24 hours, hypocalcaemia and osteoporosis are corrected with supply of Ca and D2 vitamin.

Corticotherapy is very useful in all the types of LN. Prednisone is used in doses of 1 mg/kg/day, and in severe forms 2 mg/kg/24 hours for 8 weeks, then tapered and use the alternative therapy.

In the severe forms of LN, the therapeutic scheme is represented by Methylprednisolone 1 g/kg/24 hours (pulse therapy for 3 days) followed by Prednisone orally (1 mg/kg/24 hours).

Immunosuppresive therapy is represented by:
1. Cyclophosphamide 1-2 mg/kg/day orally (12 weeks), followed by Azathioprine, or pulse therapy 0.5-1 g/m² intravenous monthly (the first 6 months), then at 3 months interval;
2. Azathioprine 2-3 mg/kg/day, 12 months;
3. Cyclosporine is effective especially for patients with pure membranous nephritis, in dose 5mg/kg/day;
4. Mycophenolate mofetil has been successfully used in severe LN (2 g/24 hours) Its efficacy is proved by studies of Chang T, Ding Lei and Belmont M.

The combination between corticotherapy and the immunosuppresives allows the dose reduction of Prednisone.

Plasmapheresis realises a fast and efficient epuration of the immune complexes, cryoglobulins, and cytotoxic antibodies.

Intravenous immunoglobulins are used when corticotherapy and immunosuppresives are not efficient.

Chronic renal failure requires dialysis or renal transplantation; arterial hypertension requires blood pressure correction with nephroprotective drugs, and edemas are managed with diuretics.

The nonsteroidal anti-inflammatory drugs reduce proteinuria.

The biological therapy with the monoclonal antibodies anti-CD40, anti CD20, anti IL10, antiCD5, immunoabsorption C1q represents the future in SLE, but the results of the studies are still not conclusive.

**MATERIAL AND METHODS**

The study was performed on 20 patients with SLE and lupus nephritis, hospitalised in Nephrology, Haemodialysis and Renal Transplantation Department of CHU Amiens France, between 2000-2004. The urine exams, urinary sediment, creatinine, clearance of creatinine, anti ds-DNA ds antibodies and renal biopsies were performed in all patients.

a. Renal biopsies examined in optic microscopy were fixed in Duboscq-Brasil and included in paraffin. The sections obtained were coloured with HPS (hematoxylin-floxin-saphran), tricrome of Masson, PAS (Schiff periodic acid). The fragments for immunofluorescence were cut and freezed with cryostat. Afterwards, the technical direct immunofluorescence with antibodies anti IgA, IgG, IgM, C3, C4, C1q and fibrinogen was performed. The renal biopsy was classified by World Health Organisation (WHO) modified in 1995:

- **Class I:** Normal glomeruli
- **Class II:** Mesangial glomerulonephritis
  - A mesangial deposits
  - B deposits and mesangial hipercellularity
- **Class III:** Proliferative segmental and focal glomerulonephritis (<50%)
  - A necrotising active lesion
  - B sclerosante and active lesions
  - C sclerosante lesions
- **Class IV:** Proliferative diffuse glomerulonephritis (>50%)
  - A without segmental lesions
  - B with necrotising active lesions
  - C with sclerosante and active lesions
  - D with sclerorotic lesions
- **Class V:** Glomerulonephritis extra membranous
  - A extra membranous pure
  - B with lesions class II
  - C with lesions class III
  - D with lesions class IV
- **Class VI:** Glomerulonephritis sclerotic

b. Index of renal involvement:
- Proteinuria (performed with colorimetric method with red of pyrogalol or blue of Coomasie, normal values (NV) < 200 mg/24 hours);
- Microalbuminuria (Imunonephelometry: NV < 20 mg/24 hours);
- Hematuria (H) and leucocituria (L) were assessed by Addis-Hamburger method, normal H< 1000 red cells/minute, L < 2000 white cells/minute;
- Serum creatinine (Jaffé method NV 50-100 µmol/l);
- Urea (NV 2.5-6.7 mmol/l);
- Clearance of creatinine with formula = U•V/P; U = urinary creatinine, P = serum creatinine, V =
urinary volume;
- Blood pressure (mmHg).

c). Biological tests: hemo-leucogram with leukocyte formula, reticulocyte, direct Coombs test.

d). Inflammatory tests: erythrocyte sedimentation rate, fibrinogen, C-reactive protein, electrophoresis of the blood proteins on agarose gel.

e). Immunological tests:
- Antinuclear antibodies – IFI on cells Hep2000 (sheets BMD) are significantly in titre over 1/100;
- Anti ds-DNA antibodies, natives, IgG – ELISA, normal < 10 UI/ml;
- Antibodies against soluble nuclear antigens: SSA, SSB, Sm, RNP, ScI70, Jo1: Radial Immunodiffusion (BMD), Immunodot (Bio Advance);
- Anticardiolipidic antibodies – ELISA (Pharmacia, serum): IgG, IgM, NV < 10 U/ml;
- Antineutrophil cytoplasmatic antibodies, ANCA, Immunofluorescence indirecte IFI (Inova): Fluorescence cytoplasmatic, c-ANCA, NV < 10; Fluorescence perinuclear, p-ANCA, VN < 10;
- Antimyeloperoxidase antibodies (Anti-MPO), ELISA (UniCap Pharmacia) NV < 7;
- Cryoglobulins- complex IgM-IgG polyclonal;
- Dosage of complement classic way (hemolise reaction: plasma on EDTA at 4 degree) CH50 NV>50%;
- Dosage of complement fractions C3c, C4, Nephelometry, C3c (NV = 0.75-1.58), C4 (NV = 0.15-0.3)
- Immunogramme: IgG (NV = 6.82-12.7), IgM (NV = 0.74-2.08), IgA (NV = 0.84-2.69).

f). Statistical method: ANOVA test with a single independently factor (the histological type) was used for the comparison.

RESULTS

The group was formed by 20 patients (18 females and 2 males), with the mean age of 37.02 ± 6.41years; the mean length of disease was 6 ± 3.12 years.

The clinical and biological parameters, recorded at the onset of LN are presented in Table 1.

The hematological modifications were represented by anemia in 75% of patients, leucopenia in 25% of patients, trombopenia in 20% of patients and positive Coombs test in 20% of patients. The inflammatory syndrome was presented in all the patients.

Electrophoresis of the seric proteins revealed low albumin in 40% of patients (2.58 ± 0.66 g/dl), hyper-alpha-2-globulinemia in 30% patients and hyper-gamma-globulinemia in all patients.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value (mean ± SD)</th>
<th>%</th>
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<tbody>
<tr>
<td>Blood pressure (mm Hg)</td>
<td>112.75 ± 16.28 / 75.09 ± 2.22</td>
<td>70%</td>
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<td></td>
<td>164.12 ± 13.25 / 94.10 ± 7.25</td>
<td>30%</td>
</tr>
<tr>
<td>Oedema</td>
<td>Absence</td>
<td>70%</td>
</tr>
<tr>
<td></td>
<td>Presence</td>
<td>30%</td>
</tr>
<tr>
<td>Serum creatinine (µmol/l)</td>
<td>81.44 ± 5.65</td>
<td>70%</td>
</tr>
<tr>
<td></td>
<td>273.75 ± 13.57</td>
<td>30%</td>
</tr>
<tr>
<td>Creatinine clearance (ml/min)</td>
<td>118.82 ± 9.27</td>
<td>50%</td>
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<td></td>
<td>41.25 ± 13.37</td>
<td>50%</td>
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<tr>
<td>Proteinuria (mg/24hours)</td>
<td>95 ± 12.14</td>
<td>5%</td>
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<td></td>
<td>3102 ± 2255</td>
<td>95%</td>
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The immunological tests in this study showed low values of complement C3c in 60% patients and C4 in 70% patients; RF (rheumatoid factor) was positive in 10% of patients;

Antinuclear antibodies were positive in 95%, and the anti ds-DNA antibodies in 90% of patients.

The antibodies against nuclear plasmatic antigenes were positive in: 20% of patients (anti-RNP), 30% of patients (anti-SSA), 5% patients (anti-SSB), 5% of patients (anti Sm), 5% of patients (ANCA); cryoglobulins were positive in 30% patients.

Immunoglobulins were elevated in 60% of patients (IgG), 10% of patients (IgM), 35% of cases (IgA).

Antiphospholipidic syndrome was present in 20% of cases.

Clinical manifestations in the studied group are presented in Table 2.

Table 2. Clinical manifestations.

<table>
<thead>
<tr>
<th>Manifestation</th>
<th>% of patients</th>
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<tbody>
<tr>
<td>Cutaneous</td>
<td>85%</td>
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<tr>
<td>Joint involvement</td>
<td>80%</td>
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<tr>
<td>Cardiovascular</td>
<td>25%</td>
</tr>
<tr>
<td>Respiratory</td>
<td>30%</td>
</tr>
<tr>
<td>Neuro-psychiatric</td>
<td>15%</td>
</tr>
<tr>
<td>Digestive</td>
<td>25%</td>
</tr>
<tr>
<td>Sjögren syndrome</td>
<td>10%</td>
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</table>

Kidney biopsies indicated the following types of glomerulonephritis: type II in 5% of patients, type III in 20 % of patients, type IV in 55% of patients and type V in 20% of patients. (Table 3)

The comparison between renal involvement indices in the different types of LN are presented in Figures 1-4.
The treatment of the LN includes the induction therapy and the maintenance therapy. In the acute phase, all the patients received corticotherapy: Methylprednisolone 1 g/24 hours, three days consecutively or Prednisone 1 mg/kg/24 hours; in all the patients was associated immunosuppressive therapy: Cyclophosphamide 1 g iv monthly, in the first 3 months, Azathoprine 100 mg/24 hours, or Mycophenolate mophetil (MMF) in progressive dose until 1500 mg/24 hours. One patient received Clorambucil 0.2 mg/kg/day (the Ponticelli protocol).

The maintenance therapy consists of corticotherapy (Prednisone) and immunosuppressive therapy (Azathioprine 100 mg/24 hours or MMF 1000 mg/24 hours).

The therapy of comorbidities was represented by Risendronate 35 mg/day and Calcium + D3 2 cp/day (for osteoporosis), calcium blockers (for hypertension), Omeprazole 20 mg/day (for gastroduodenitis due to cortisone), anticoagulant treatment with Enoxaparine or Fluindione in the antiphospholipidic syndrome. The contraception was made only with progesterone: Luteran.

The evolution of the studied patients was favourable in 80% cases, with normalisation of creatinine, urea, and reduction of proteinuria. In 20% of patients, the evolution was progressive to the end stage of renal disease (ESRD).

**DISCUSSION**

SLE represents a multisystemic disease, with autoimmune pathogenesis (circulating immune complexes CIC). The kidney is the main target of CIC. The disease is more frequent among females. In our study, the female/man ratio was high (9/1), similar with other studies. The mean average duration of SLE was of 6 ± 3.12 years. Oral contraception was found in 10% of patients and spontaneous miscarriage in 20% of cases.

The kidney involvement in SLE is frequent, with many modalities of evolution. Analysing the clinical and the biological signs of the renal involvement at onset we have found a strong association between the values of proteinuria, hematuria, and the hypertension with a worse class of LN, in concordance with the study of Zappitelli. The minimal values of proteinuria were found in type II of LN and the highest values of proteinuria were found in type V of LN. (Fig 1) Hematuria was found in all types of LN. (Fig 2) The highest values of serum creatinine and the reduced levels of creatinine...
clearance appeared in type IV of LN. (Figs. 3, 4)

We didn't find a correlation between the RF and LN similar with the study of Helin and in contrast with the study of Miyazaki et al, which have found a high titre of RF in the serum of patients with proliferative LN.14,15

The anti ds-DNA antibodies are highly specific for SLE and they are positive in 90% of patients, similar to the other studies.1,16 The titre is correlated with disease activity. The detection is important for the diagnosis and for the clinical and therapeutical monitoring.

In contrast with other studies, the prevalence of the anti-Sm antibodies is low 5%: also the ANCA antibodies 5% and there is no correlation with LN or with the deterioration of the renal function.1,17,18 The prevalence of the antiphospholipidic antibodies was 20%, without association with more severe LN in concordance with other studies.17,19 Similarly to the other studies, we have found a secondary Sjögren syndrome in 10% patients, without correlation with LN.20

The evolution of patients under treatment was favourable in 80% of cases the proteinuria (p < 0.05), the serum creatinine and the creatinine clearance improved after 6 months of treatment. Immunosuppressive regimes (Prednisone + Cyclophosphamide vs. Prednisone + MMF) had a similar efficiency in induction and maintain of remission in the studied patients.

In 20% patients, despite the sustained treatment, the evolution was not favourable, with the progression to the ESRD. All these patients had type IV of LN.

The role of the hormonal factors is well known in lupus, suggested by the predominance of the disease in women. The estrogens are involved in induction and in worsening of the lupus. In the light of the recent studies, Sanchez Guerrero et al and Petri concern usage of oral contraceptives in women with SLE, there is no difference between the combination of estro-progestatives and progestatives alone in terms of disease activity, the incidence of flares, adverse effects.21,22 In our study contraception is done with estro-progestatives and progestatives alone in terms of disease activity. The detection is important for the diagnosis and for the clinical and therapeutical monitoring.

CONCLUSION

All the patients with favourable evolution achieved clinical remission that demonstrated the importance of the earlier diagnosis of LN by kidney biopsy and the earlier starting of treatment with pulse therapy Cyclophosphamide combined with Prednisone orally or intravenous in according to the results of the clinical trials (NIH in USA and Eurolupus trial in Europe).23,24,25

The combination mycophenolate mofetil with Prednisone is also efficient, both in induction therapy and in maintenance, similarly to the previous studies.6,7

As negative prognosis factors we have found: younger age at diagnosis, the presence of hypertension, persistence of hematuria, high titre of anti-ds DNA antibodies and lower complement, despite the treatment.

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REFERENCES