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# Deposition of the lectin pathway of complement in renal biopsies of lupus nephritis patients

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## ABSTRACT

**Background/aims:** Lupus nephritis (LN) is one of the most serious manifestations of SLE occurring in 66–90% of these patients. The complement system is part of the innate immunity and modulator of inflammation and the adaptative immune response. Mannan-binding lectin (MBL) and Ficolin-2 (FCN-2) are important members of the lectin pathway of complement activation. Despite the significant participation of complement in the pathogenesis of the LN, there are few reports demonstrating “*in situ*” deposition of complement components in renal biopsy specimens in this disorder. The present study investigated the deposition of complement components in kidney specimens of LN patients.

**Methods:** Renal biopsies of 11 patients with SLE and LN were evaluated for immunofluorescence staining for IgG, IgA, IgM, C3, and C1q. Additionally, MBL, FCN-2 and C5b-9 were researched using monoclonal antibodies.

**Results:** All the biopsies were positive for IgG, C3, and C1q, eight were positive IgM and five had IgA deposition in glomerular tissue. The terminal complex of complement C5b9 was positive in all cases, MBL in nine (82%) cases; seven (63.6%) of them presenting concomitantly FCN-2 deposition. Patients presenting MBL deposition had higher mean of urinary proteins (9.0 g/day) than patients with negative MBL deposition (mean of 2.3 g/day).

**Conclusions:** In this study, we demonstrated *in situ* the participation of complement in the renal injury, including MBL and FCN-2 of the lectin pathway; also the strong role of C5b-9 in the pathogenesis of LN.

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## 1. Introduction

Systemic lupus erythematosus (SLE) is a multisystemic disease, which is associated with the production of autoantibodies and immune complexes formation, which predominantly target nuclear antigens. Lupus nephritis (LN) remains a major challenge and continues to be one the most severe manifestation of SLE, occurring in 50–80% of these patients [1]. The morphologic findings of LN in renal biopsy comprise a spectrum of vascular, glomerular and tubulointerstitial lesions [1]. LN is histologically evident in most patients with SLE, even those without clinical manifestations of renal disease. The progression of LN can be related to hypertension, proteinuria and renal failure. In the direct immunofluorescence, IgG deposition is found positive in more than 90% of cases; IgA and IgM staining in 60–70% of cases; C3 and C1q in around 80% of cases [2]. The presence of the three immunoglobulin (Igs) with

C3 and C1q is denominated as “full house” pattern, characterizing LN and uncommon in other renal diseases. The presence of subendothelial deposits in glomerular capillaries is crucial in the induction of severe damage [3]. However, the clinical presentation does not correlate very well with the type and severity of renal biopsy histology findings [1]. Based on experimental models of autoimmune and immune complex disease in the kidney and on observations in human renal biopsies, it is well established that the glomerular patterns of immune complex-mediated injury are related to the site of accumulation of immunoglobulin, their antigen specificity, their capacity to bind and activate complement and other serine proteases as well as their ability to evoke a cellular inflammatory response [4]. Findings of hypocomplementemia in most of patients with active disease, and, at least in some, complement levels correlate with the activity of renal disease [5].

The complement system is part of the innate immunity and an important modulator of inflammation and the adaptative immune response. Mannan-binding lectin (MBL) and Ficolin-2 (FCN-2) are important members of the lectin pathway of complement activation. MBL is a C-type plasma lectin acting as an ante-antibody and/or as a disease modifier molecule [6]. MBL is able to eliminate

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potential pathogenic organisms by activating the complement cascade and by facilitating phagocytosis as an opsonin. In addition, it has been shown that MBL is able to promote direct opsonophagocytosis, clearance of apoptotic cells and to modulate inflammation [7]. High levels of MBL and related genotypes have been shown associated with disease severity in chronic inflammatory conditions [7,8]. Takahashi et al. [9] described that *MBL2* gene polymorphism influences susceptibility to SLE, but has no direct effect on disease characteristics and that serum MBL concentrations vary during the course of SLE. FCN-2 are pattern-recognition proteins capable to bind to specific pathogen-associated molecular patterns (PAMPs) on microorganism surfaces, triggering the innate immune response by either binding to collectin cellular receptors or activating the complement lectin pathway [10]. Watanabe et al. [11] related significant decreased FCN-2 levels in the serum of SLE patients, however, not associated with activity of disease or C3 and C4 levels.

Despite the significant participation of complement in the pathogenesis of the LN, there are few reports demonstrating “*in situ*” deposition of complement components in renal biopsy specimens in this disorder. Sato et al. [12] showed that complement pathway activation is a basis of LN pathogenesis and that alternative as well as lectin pathway are involved in the progression of glomerular injury of Japanese patients with SLE and LN.

Considering the central role of complement in renal injury in patients with LN, the present study investigated the deposition of complement components in kidney specimens of LN patients from South Brazil.

## 2. Materials and methods

The local Research Ethics Committee approved the study.

Eleven patients (eight female and three male, between 23 and 41 years old) diagnosis with SLE according to American College of Rheumatology [13] were included in the study. Renal biopsies that were used for routine diagnostic of LN and kept cryopreserved at  $-80^{\circ}\text{C}$  of until used. The diagnosis of LN was based on clinical and histological evaluation of renal biopsy tissue. For light microscopy, paraffin-embedded sections were routinely stained with hematoxylin and eosin, periodic acid–Schiff, AZAN or Masson trichrome and periodic acid–silver methenamine. Two experienced pathologists evaluated these sections. Histological evaluation was divided into groups according to the International Society of Nephrology (ISN)/Renal Pathology Society (RPS) WHO classification [14]. For routine immunofluorescence staining for IgG, IgA, IgM, C3, and C1q was performed on fresh frozen renal specimens was using corresponding fluorescein isothiocyanate (FITC) conjugated antibodies (polyclonal antibody, dilution 1/40, Dako, Copenhagen, Denmark). Kidney tissues of five autopsies from individuals without renal disease were used as negative controls.

All specimens were cut in cryostat in  $4\mu\text{m}$  thick sections and placed on albumin treated slides. For the detection of MBL, FCN-2, and C5b-9, the slides with the cryostatic skin sections were incubated overnight at  $4^{\circ}\text{C}$  with the monoclonal antibody diluted in PBS buffer pH 7.4 (*anti-MBL*, Mo Hyb 131-01, diluted 1/50, Bio-Porto, Gentofte, Denmark; *anti Ficolin-2*, Mo P-35, diluted 1/20, Hy-cult, Uden, The Netherlands and *anti C5b-9*, Mo w13-15, diluted 1/20, kindly provided as a gift from Prof. Dr. RW), respectively. After incubation, the slides were washed for three times with PBS pH 7.4 and incubated with conjugated antibody (polyclonal goat anti-Mouse/FITC (Sigma, San Diego, USA) diluted 1/200 at room temperature for 60 min. For the detection of C3, IgG, IgA, IgM, and C1q, diluted polyclonal antibodies were applied to the skin sections and incubated for 60 min at room temperature. After washed for three

times with buffer, the slides were mounted with glycerin alkaline and observed in fluorescence microscope (Olympus, Tokyo, Japan) using specific software to capture image. The intensity of all positive deposits was evaluated by two blinded independent observers and graded as follows: N = negative staining, L = minimal staining, M = moderate staining and S = strong staining.

On the same day which the patient has been biopsied, data from laboratory measurements of urinary proteins (g/day), creatinine clearance, serum creatinine, serum concentrations of C3 and C4, antinuclear and anti-DNA antibodies were collected.

## 3. Results

The findings of immunofluorescence deposition in patients with LN are demonstrated in Table 1 and in Fig. 1. All the biopsies ( $n = 11$ ) were positive for IgG, C3, and C1q, with most of them showing intense pattern of deposition in capillaries. Eight (73%) patients showed deposition of IgM and five (45%) had IgA deposition in glomerular tissue. The “full-house” pattern was observed in 4 (36%) patients.

The terminal complex of complement C5b9 was positive in all cases, with different degree of intensity. MBL deposition was positive in nine (82%) cases; seven (63.6%) of them presenting concomitantly FCN-2 deposition. In two cases, both components of the lectin pathway were negative. MBL and C5b-9 were deposited in different areas in the renal tissue, including glomeruli, mesangial cells, blood vessels, tubular tissue and Bowman’s capsule. Additionally, it was observed that patients presenting MBL deposition had higher mean of urinary proteins (9.0 g/day) than patients with negative MBL deposition (mean of 2.3 g/day). None of the other laboratory parameters were associated with different degree and/or pattern of deposition.

In the biopsies of controls, no positivity was observed for all investigated markers.

## 4. Discussion

LN carries significant morbidity, mortality and the renal involvement is a key determinant for the prognosis of SLE. The prognosis of LN depends on a large number of demographic, racial, genetic, histopathological, immunological and time-dependent factors [15].

The complement system plays a key role as a humoral component of the innate immune system and also starting the specific immune response. On the other hand, when inappropriately activated or regulated, complement can cause inflammation and tissue damage. Complement is involved in the pathogenesis of different

**Table 1**  
Findings of immunofluorescence deposition in patients with lupus nephritis.

Patient number	Class	IgA	IgM	IgG	C3	C1q	C5b9	MBL	2-Ficolin
1	Class II	Neg	L	S	S	S	S*	S	M
2	Class II	M	M	S	S	S	S*	S	M
3	Class III	L	M	S	S	S	M	M	M
4	Class III	L	L	M	M	M	L	L	L
5	Class III	L	Neg	M	M	M	M	Neg	Neg
6	Class IV	Neg	S	S	S	S	S	S	M
7	Class IV	Neg	M	S	S	S	M	M	M
8	Class IV	M	L	S	S	S	S#	S	M
9	Class V	Neg	Neg	S	L	L	L	Neg	Neg
10	Class V	M	L	S	S	S	M	M	Neg
11	Class V	Neg	S	S	S	S	M	M	Neg

Neg = Negative; S = Severe; M = Moderate; L = Low.

\* Moderate deposition of C5b9 also in blood vessels; #Deposition of C5b9 also in “Bowman’s capsule”.

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