BRIEF COMMUNICATION

Anti-C1q, anti-chromatin/nucleosome, and anti-dsDNA antibodies in juvenile systemic lupus erythematosus patients

Adriana Almeida de Jesus[1], Lucia Maria Arruda Campos[1], Bernadete Lourdes Liphaus[2], Magda Carneiro-Sampaio[3], Cristóvão Luis Pitangueira Mangueira[4], Eliane Aparecida Rosseto[5], Clovis Artur Almeida da Silva[6], Morton Scheinberg[7]

ABSTRACT

Objectives: To evaluate the presence of anti-C1q, anti-chromatin/nucleosome and anti-double stranded DNA (dsDNA) antibodies in juvenile systemic lupus erythematosus (JSLE) and controls. **Methods:** Sixty-seven JSLE and 34 healthy controls were analyzed for the presence of anti-C1q, anti-chromatin/nucleosome, and anti-dsDNA antibodies by ELISA. C1q levels were evaluated by radial immunodiffusion. **Results:** The mean current age was similar in JSLE patients and controls (14.6 ± 3.86 *vs.* 13.6 ± 2.93 years, P = 0.14). Higher frequencies of anti-C1q, anti-chromatin/nucleossome, and anti-dsDNA antibodies were observed in JSLE compared to controls (20% *vs.* 0%, P = 0.0037; 48% *vs.* 0%, P < 0.0001 and 69% *vs.* 3%, P < 0.0001, respectively). The median of anti-C1q, anti-chromatin/nucleossome, and anti-dsDNA antibodies were also significantly higher in JSLE patients than in controls [9.6 (5.5–127) *vs.* 7.5 (5–20) units, P = 0.0006; 18 (1.9–212) *vs.* 3.2 (1.7–17) units, P < 0.0001; and 111 IU/mL (6–741) *vs.* 14 (6–33) IU/mL; P < 0.0001, respectively]. The sensitivity for anti-C1q, anti-chromatin/nucleosome, and anti-dsDNA antibodies was 21% (CI: 11–33), 49% (CI: 36–62), and 70% (CI: 57–81). The specificity was 100% (CI: 88–100), 100% (88–100), and 97% (CI: 83–99), respectively. A positive correlation was found between anti-dsDNA levels and both anti-C1q (r = 0.51; CI: 0.29–0.68; P < 0.0001) and anti-chromatin/nucleosome antibodies (r = 0.87; CI: 0.79–0.92; P < 0.0001) levels. A negative correlation was observed between anti-C1q and C1q levels (r = −0.33; CI: −0.56–0.05; P = 0.018). The frequency of anti-dsDNA was higher in patients with SLEDAI-2K ≥1 (P = 0.0047) and no differences were observed in the frequencies of these three autoantibodies and nephritis (P > 0.05). **Conclusion:** Our study demonstrated an elevated specificity for lupus diagnosis involving the three autoantibodies, especially anti-C1q and anti-chromatin/nucleosome.

Keywords: juvenile systemic lupus erythematosus, complement C1q, nucleosomes, autoantibodies, DNA.

INTRODUCTION

Systemic lupus erythematosus (SLE) is a chronic inflammatory disease characterized by the presence of multiple autoantibodies. Specific and pathogenic autoantibodies were previously studied in juvenile SLE (JSLE) patients, particularly anti-C1q,[1–4] anti-double stranded DNA (anti-dsDNA),[3–5] and anti-chromatin/nucleosome antibodies.[4,5] We have previously shown that in JSLE patients the presence of these autoantibodies were associated with lupus activity[5] and may be a

valuable tool for monitoring the disease course.[3] However, to our knowledge, the concomitant evaluation of these three antibodies with assessments of sensitivity, specificity, and positive and negative predictive values for JSLE diagnosis were not carried out in a pediatric lupus population.

Therefore, we evaluated the prevalence of anti-C1q, anti-dsDNA, and anti-chromatin/nucleosome antibodies in JSLE patients and in controls and the possible association of these antibodies with lupus nephritis and disease activity. In addition, we assessed sensitivity, specificity, and positive and negative predictive values of the three autoantibodies for JSLE diagnosis.

MATERIALS AND METHODS

Sixty-seven consecutive JSLE patients followed at the Pediatric Rheumatology Unit were evaluated. All patients fulfilled the American College of Rheumatology (ACR) SLE classification criteria.[6] The control group included 34 healthy subjects followed at the Adolescents Unit at the same University Hospital. The Local Ethical Committee approved this study and an informed consent was obtained from all participants.

Autoantibodies and C1q assessments

Anti-C1q antibodies were detected by enzyme-linked immunosorbent assay (ELISA) (Inova Diagnostics – QUANTA Lite™ Anti-C1q, San Diego, CA, USA). The cut-off for a positive test result was 20 units, as determined by the manufacturer. Anti-chromatin/nucleossome antibodies were determined by ELISA (Inova Diagnostics – QUANTA Lite™ Anti-nucleossome, San Diego, California, USA). The cut-off for a positive test result was 20 units, as also determined by the manufacturer. Anti-dsDNA antibodies were detected by Farrzyme assay (The Binding Site, Birmingham, UK) with cut-off of 30 units, as determined by the manufacturer. All antibodies were assessed with duplicated samples in JSLE patients and controls. C1q levels were evaluated by radial immunodiffusion (The Binding Site, Birmingham, UK) in JSLE patients and the normal values were 33−209 mg/L.

Demographic, nephritis, and disease activity data evaluation

Demographic data included current age, age at JSLE onset and gender. Renal involvement was defined according to proteinuria[3] 0.5 g/24h, presence of cellular casts or persistent hematuria[3] 10 red blood cells per high power field. SLE disease activity at the time of study entry was measured in all patients, using the SLE Disease Activity Index 2000 (SLEDAI-2K).[7]

Disease activity was defined as SLEDAI-2K $\geq$ 1 and the following activity categories have been considered according to SLEDAI-2K score: no activity (SLEDAI-2K = 0), mild activity (SLEDAI-2K = 1–5), moderate activity (SLEDAI-2K = 6–10), high activity (SLEDAI-2K = 11–19), and very high activity (SLEDAI-2K = 20).[8]

Statistical analysis

Results were presented as the mean ± standard deviation (SD) or median for continuous variables, and number (%) for categorical variables. Data were compared by *t* test in continuous variables to evaluate differences between JSLE and controls, and in JSLE subgroups. Categorical variables differences were assessed by Fisher's exact test. Spearman's coefficient was used to evaluate correlations between serum autoantibodies, and between anti-C1q antibodies and C1q levels. The sensitivity, specificity, and positive and negative predictive values of these antibodies for JSLE diagnosis were also evaluated. In all the statistical tests the level of significance was set at 5% ($P < 0.05$).

RESULTS

The mean current age was comparable in JSLE patients and controls (14.6 ± 3.86 *vs.* 13.6 ± 2.93 years; P = 0.14) and the percentage of female gender was similar in both groups (83% *vs.* 79%, P = 0.58) (Table 1). The age at JSLE onset and the disease duration were 8.9 ± 3.20 and 6.4 ± 3.52 years, respectively.

Higher frequencies of elevated anti-C1q, anti-chromatin/nucleosome and anti-dsDNA antibodies were observed in JSLE

Table 1
Demographic data and anti-C1q, anti-chromatin/nucleosome and anti-dsDNA autoantibodies in juvenile systemic lupus erythematosus patients and controls

Variables	JSLE (n = 67)	Controls (n = 34)	P
Demographic data			
Age at disease onset, years	8.9 ± 3.20	—	—
Disease duration, years	6.4 ± 3.52	—	—
Current age, years	14.6 ± 3.86	13.6 ± 2.93	0.14
Female gender	52 (83)	27 (79)	0.58
Autoantibodies			
Anti-C1q, units	9.6 (5.5–127)	7.5 (5–20)	0.0006
Elevated levels (> 20 U)	13 (20%)	0 (0%)	0.0037
Anti-chromatin/ nucleosome, units	18 (1.9–212)	3.2 (1.7–17)	< 0.0001
Elevated levels (> 20 U)	30 (48%)	0 (0%)	< 0.0001
Anti-dsDNA, IU/mL	111 (6–741)	14 (6–33)	< 0.0001
Elevated levels (> 30 IU)	43 (69%)	1 (3%)	< 0.0001

compared to controls (20% *vs*. 0%, P = 0.0037; 48% *vs*. 0%, P < 0.0001; and 69% *vs*. 3%, P < 0.0001, respectively). The medians of anti-C1q, anti-chromatin/nucleossome, and anti-dsDNA antibodies were also significantly higher in JSLE patients than in controls [9.6 (5.5–127) *vs*. 7.5 (5–20) units, P = 0.0006; 18 (1.9–212) *vs*. 3.2 (1.7–17) units, P < 0.0001; and 111 (6–741) *vs*. 14 (6–33) IU/mL, P < 0.0001, respectively] (Table 1).

The sensitivities for anti-C1q, anti-chromatin/nucleossome, and anti-dsDNA antibodies were 21% (confidence interval (CI): 11%–33%, P = 0.0037), 49% (CI: 36%–62%; P < 0.0001), and 70% (CI: 57%–81%; P < 0.0001); and the specificities were 100% (CI: 88–100%; P = 0.0037), 100% (CI: 88–100%; P < 0.0001), and 97% (CI: 83–99%; P < 0.0001), respectively. Positive predictive values were 100% (CI: 75%–100%; P = 0.0037), 100% (CI: 88%–100%; P < 0.0001), and 97% (CI: 87%–99%; P < 0.0001), and negative predictive values were 39% (CI: 28%–50%; P = 0.0037), 50% (CI: 37%–62%; P < 0.0001), and 62% (CI: 47%–76%; P < 0.0001) for anti-C1q, anti-chromatin/nucleossome, and anti-dsDNA antibodies, respectively.

Nephritis was evidenced in 50 (81%) of JSLE patients. No differences were observed in JSLE patients with and without nephritis in the frequencies of anti-C1q (26% *vs*. 8%, P = 0.26), anti-chromatin/nucleosome (48% *vs*. 50%; P = 1.00), and anti-dsDNA antibodies (72% *vs*. 58%; P = 0.30).

Disease activity was observed in 48 (77%) of JSLE patients. No differences were observed in JSLE patients with SLEDAI ≥ 1 *vs*. SLEDAI < 1 (SLEDAI = 0) in the frequencies of anti-C1q (20% *vs*. 21%; P = 1.00) and anti-chromatin/nucleosome (45% *vs*. 21%; P = 0.13). On the other hand, the frequency of anti-dsDNA antibodies was significantly higher in patients with SLEDAI ≥ 1 *vs*. SLEDAI = 0 (51% *vs*. 7%; P = 0.0047).

In addition, the median of SLEDAI-2K was significantly higher in patients with positive anti-dsDNA in comparison with patients with negative anti-dsDNA [8 (0–18 UI/mL) *vs*. 4 (0–16 UI/mL); P = 0.004]. Even though, the medians of SLEDAI-2K were similar in patients with positive and negative anti-chromatin/nucleosome [6 (0–16) *vs*. 4 (0–18); P = 0.11] and in patients with positive and negative anti-C1q antibodies [5 (0–18) *vs*. 4 (0–16); P = 0.86].

According to disease activity, the patients were classified in five groups: no activity (SLEDAI-2K = 0) (n = 14; 21%); mild activity (SLEDAI-2K = 1–5) (n = 21; 31%); moderate activity (SLEDAI-2K = 6–10) (n = 18; 27%); high activity (SLEDAI-2K = 11–19) (n = 14; 21%) and very high activity (SLEDAI-2K ≥ 20 (n = 0; 0%).

The frequency of anti-dsDNA antibodies was significantly lower in patients with no activity in comparison with patients with mild (14% *vs*. 52%; P = 0.033), moderate (14% *vs*. 61%; P = 0.011),

and high disease activity (14% *vs*. 79%; P = 0.0018). No difference was observed between the frequency of anti-dsDNA antibody in patients with mild and moderate activity (52% *vs*. 61%; P = 0.74) and mild and high activity (52% *vs*. 79%; P = 0.16) and between moderate and high activity patients (61% *vs*. 79%; P = 0.16).

Regarding the anti-chromatin/nucleosome antibodies, patients with moderate activity presented a higher frequency of this antibody than patients with mild activity (61% *vs*. 24%; P = 0.025) and with no activity (61% *vs*. 21%; P = 0.035). The frequency of anti-chromatin/nucleosome antibody was similar between patients with no activity and patients with mild (21% *vs*. 24%; P = 1.00) and high activity (21% *vs*. 50%; P = 0.23) and between patients with mild and high disease activity (24% *vs*. 50%; P = 0.15).

Regarding anti-C1q antibody, patients with no disease activity presented the same frequency of anti-C1q antibodies as patients with mild (21% *vs*. 24%; P = 1.00), moderate (21% *vs*. 17%; P = 1.00), and high (21% *vs*. 29%; P = 1.00) disease activities. Moreover, no difference was observed between the frequency of anti-C1q antibodies in patients with mild activity compared to moderate (24% *vs*. 17%; P = 0.70) and high (24% *vs*. 29%; P = 1.00) disease activity and in patients with moderate activity compared to high disease activity (17% *vs*. 29%; P = 0.66).

Figure 1 shows the frequency of anti-dsDNA, anti-chromatin/nucleosome, and anti-C1q antibodies according to different degrees of disease activity.
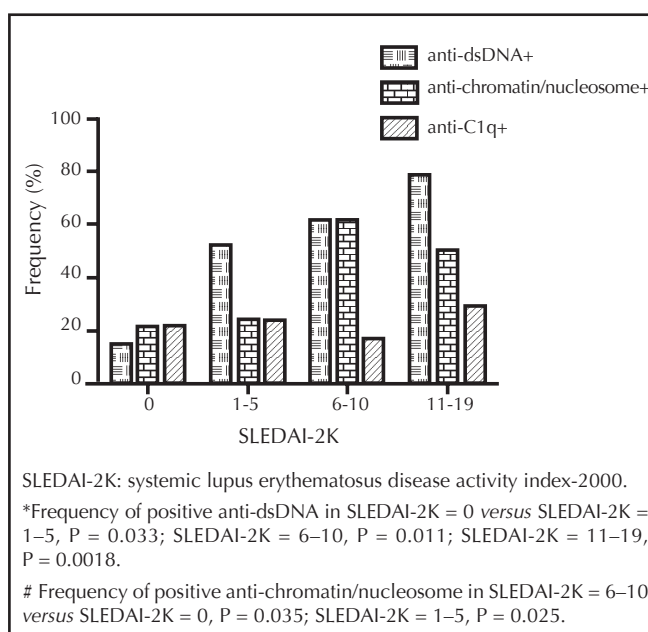

SLEDAI-2K: systemic lupus erythematosus disease activity index-2000.

*Frequency of positive anti-dsDNA in SLEDAI-2K = 0 *versus* SLEDAI-2K = 1–5, P = 0.033; SLEDAI-2K = 6–10, P = 0.011; SLEDAI-2K = 11–19, P = 0.0018.

Frequency of positive anti-chromatin/nucleosome in SLEDAI-2K = 6–10 *versus* SLEDAI-2K = 0, P = 0.035; SLEDAI-2K = 1–5, P = 0.025.

Figure 1
Frequency of anti-dsDNA, anti-chromatin/nucleosome and anti-C1q antibodies according to SLE disease activity (SLEDAI-2K).

Regarding the discordance between the three autoantibodies, remarkably, one patient was positive only for anti-C1q antibody (negative for anti-chromatin/nucleosome and anti-dsDNA antibodies) and another patient was positive only for anti-chromatin/nucleosome antibody (negative for anti-C1q and anti-dsDNA autoantibodies).

Figure 2 shows a positive correlation between anti-C1q and anti-dsDNA antibodies in JSLE patients (r = 0.51; IC: 0.29–0.68; P < 0.0001). Figure 3 demonstrates a positive correlation between anti-chromatin/nucleosome and anti-dsDNA antibodies in JSLE patients (r = 0.87; CI: 0.79–0.92; P < 0.0001).
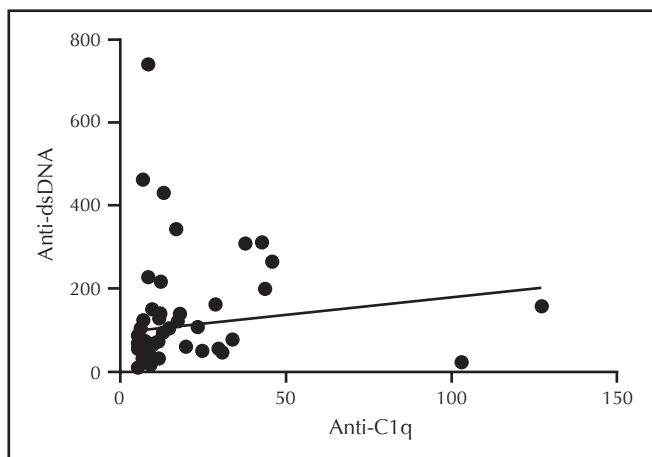

Figure 2
Positive correlation between anti-C1q and anti-dsDNA antibodies in 62 juvenile systemic lupus erythematosus patients (r = 0.51; IC: 0.29–0.68; P = 0.0001).
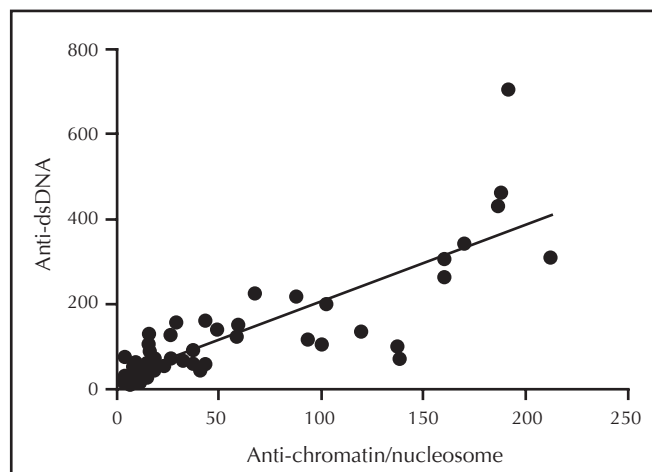

Figure 3
Positive correlation between anti-chromatin/nucleosome and anti-dsDNA antibodies in 62 juvenile systemic lupus erythematosus patients (r = 0.87; CI: 0.79–0.92; P = 0.0001).

In addition, a negative correlation was observed between anti-C1q and C1q serum levels (r = −0.33; CI: −0.56–0.05; P = 0.018). None of them had undetectable C1q levels compatible with a primary C1q immunodeficiency.

DISCUSSION

Our study demonstrated an elevated specificity and positive predictive value for lupus diagnosis of these three autoantibodies, especially anti-chromatin/nucleosome and anti-C1q antibodies. In addition, anti-dsDNA antibody was considered a reliable marker of disease activity in our patients and the anti-chromatin/nucleosome antibody showed a fairly strong positive correlation with anti-dsDNA and a higher frequency in moderate activity patients in comparison to no active and mildly active patients.

Anti-C1q antibodies have been associated to lupus nephritis and disease activity in adult[9–12] and juvenile lupus patients.[4] However, we recently demonstrated that this antibody in our cohort was not associated with nephritis in 67 JSLE patients,[3] as observed herein. Likewise, Ravelli et al.[1] observed no association between anti-C1q levels and renal involvement. Nonetheless, the present study showed a positive correlation between anti-C1q and anti-dsDNA antibodies. The latter is a well-known biomarker of disease activity of SLE, as evidenced herein. Another important consideration is that a standardized set of reagents for the determination of anti-C1q antibodies has not been established yet, and this may partially explain some discrepancies in the previous results.

Additionally, a negative correlation was observed between anti-C1q and C1q serum levels in our patients, as also evidenced in a Chinese study with pediatric lupus patients.[4] Therefore, the presence of these antibodies could lead to a secondary decrease in C1q levels and to an impairment of autoantigens clearance, contributing to lupus pathogenesis.[13,14]

Anti-chromatin/nucleosome antibodies have also been described as a marker of disease activity and lupus nephritis in adult patients.[15–18] It has been demonstrated *in vitro* a direct binding of both C1q and nucleosomes to glomerular endothelial cells and that anti-nucleosome and anti-C1q antibodies have an additive effect in lupus nephritis pathogenesis in adults.[19] Regarding pediatric lupus, our group previously showed that the presence of this antibody was associated with lupus activity but not with renal manifestations,[2] as also observed in the current study, which showed that this antibody might be a useful marker of moderately active disease.

Importantly, these autoantibodies had a markedly high specificity and positive predictive value, greater than 97%,

for lupus diagnosis, and can be considered as a reliable tool in clinical practice, especially anti-chromatin/nucleosome and anti-C1q antibodies. Indeed, in previous studies, the specificity and positive predictive value for anti-chromatin/nucleosome antibodies for JSLE diagnosis were reported from 96%–98%[4,5] and 97%,[5] respectively. Regarding the anti-C1q antibodies, the specificity for JSLE diagnosis was 92%–100%.[3,4]

The determinations of anti-chromatin/nucleosome and anti-C1q antibodies should be carried out for evaluation of lupus diagnosis, especially in JSLE patients who are negative for anti-dsDNA autoantibodies. These exams may be considered as lupus biomarkers, particularly in patients with incomplete lupus (up to three ACR classification lupus criteria) and prospective studies are necessary.

In conclusion, although the anti-C1q and anti-nucleosome autoantibodies presented a lower sensitivity compared to anti-dsDNA, the exceedingly high specificity and positive predictive value of both antibodies could help in JSLE diagnosis, especially in patients with negative anti-dsDNA.

REFERENCES

REFERÊNCIAS

1. Ravelli A, Wisnieski JJ, Ramenghi B, Ballardini G, Zonta L, Martini A. IgG autoantibodies to complement C1q in pediatric-onset systemic lupus erythematosus. Clin Exp Rheumatol 1997; 15(2):215–19.

2. Kozyro I, Perahud I, Sadallah S, Sukalo A, Titov L, Schifferli J *et al*. Clinical value of autoantibodies against C1q in children with glomerulonephritis. Pediatrics 2006; 117(5):1663–8.

3. Jesus AA, Silva CA, Carneiro-Sampaio M, Sheinberg M, Mangueira CL, Marie SK *et al*. Anti-C1q antibodies in juvenile-onset systemic lupus erythematosus. Ann N Y Acad Sci 2009; 1173:235–8.

4. Wu FQ, Zhao Q, Cui XD, Zhang W. C1q and anti-C1q antibody levels are correlated with disease severity in Chinese pediatric systemic lupus erythematosus. Rheumatol Int 2011; 31(4):501–5.

5. Campos LM, Kiss MH, Scheinberg MA, Mangueira CL, Silva CA. Antinucleossomo antibodies in patients with juvenile systemic lupus erythematosus. Lupus 2006; 15(8):496–500.

6. Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erhytematosus. Arthritis Rheum 1997; 40(9):1725.

7. Gladman DD, Ibañez D, Urowitz MB. Systemic lupus erythematosus disease activity index 2000. J Rheumatol 2002; 29(2):288–91.

8. Griffiths B, Mosca M, Gordon C. Assessment of patients with systemic lupus erythematosus and the use of lupus disease activity indices. Best Pract Res Clin Rheumatol 2005; 19(5):685–708.

9. Trendelenburg M, Lopez-Trascasa M, Potlukova E, Moll S, Regenass S, Frémeaux-Bacchi V *et al*. High prevalence of anti-C1q antibodies in biopsy-proven active lupus nephritis. Nephrol Dial Transplant 2006; 21(11):3115–21.

10. Sinico RA, Rimoldi L, Radice A, Bianchi L, Gallelli B, Moroni G. Anti-C1q autoantibodies in lupus nephritis: prevalence and clinical significance. Ann N Y Acad Sci 2005; 1050:193–200.

11. Katsumata Y, Miyake K, Kawaguchi Y, Okamoto Y, Kawamoto M, Gono T *et al*. Anti-C1q antibodies are associated with systemic lupus erythematosus global activity but not specifically with nephritis: a controlled study of 126 consecutive patients. Arthritis Rheum 2011; 63(8):2436–44.

12. Akhter E, Burlingame RW, Seaman AL, Magder L, Petri M. Anti-C1q antibodies have higher correlation with flares of lupus nephritis than other serum markers. Lupus 2011; 20(12):1267–74.

13. Carneiro-Sampaio M, Liphaus BL, Jesus AA, Silva CA, Oliveira JB, Kiss MH. Understanding systemic lupus erythematosus physiopathology in the light of primary immunodeficiencies. J Clin Immunol 2008; 28(Suppl 1):S34–41.

14. Flierman R, Daha MR. Pathogenic role of anti-C1q autoantibodies in the development of lupus nephritis − a hypothesis. Mol Immunol 2007; 44(1-3):133–8.

15. Kiss E, Lakos G, Szegedi G, Poor G, Szodoray P. Anti-nucleossomo antibody, a reliable indicator for lupus nephritis. Autoimmunity 2009; 42(5):393–8.

16. Muller S, Dieker J, Tincani A, Meroni PL. Pathogenic anti-nucleossomo antibodies. Lupus 2008; 17(5):431–6.

17. Gómez-Puerta JA, Burlingame RW, Cervera R. Anticromatina (anti-nucleossomo) antibodies: diagnostic and clinical value. Autoimmun Rev 2008; 7(8):606–11.

18. Souza A, da Silva LM, Oliveira FR, Roselino AM, Louzada-Junior P. Anti-nucleossomo and anticromatina antibodies are present in active systemic lupus erythematosus but not in the cutaneous form of the disease. Lupus 2009; 18(3):223–9.

19. O'Flynn J, Flierman R, van der Pol P, Rops A, Satchell SC, Mathieson PW *et al*. Nucleossomos and C1q bound to glomerular endothelial cells serve as targets for autoantibodies and determine complement activation. Mol Immunol 2011; 49(1-2):75–83.