SOCIEDADE BRASILEIRA DE REUMATOLOGIA

REVISTA BRASILEIRA DE REUMATOLOGIA

www.reumatologia.com.br

Original article

# Hepatitis C virus antibodies in high risk juvenile onset systemic lupus erythematosus

*Nádia E. Aikawa*[a,b,*], *Ana P. Nascimento*[a], *André L.S. Hayata*[a], *Eloisa Bonfá*[a], *Cláudia Goldenstein-Schainberg*[a]

[a] *Division of Rheumatology, Universidade de São Paulo, São Paulo, SP, Brazil*
[b] *Pediatric Rheumatology Unit, Hospital da Criança, Hospital das Clínicas, School of Medicine, Universidade de São Paulo, São Paulo, SP, Brazil*

ARTICLE INFO

*Article history:*
Received 22 October 2014
Accepted 27 October 2015
Available online 19 March 2016

*Keywords:*
Hepatitis C virus
Anti-HCV
Juvenile systemic lupus erythematosus

ABSTRACT

*Objective:* To evaluate the prevalence of hepatitis C virus (HCV) infection in high risk juvenile systemic lupus erythematosus (JSLE).
*Study design:* Forty low income JSLE patients (6M:34F; mean age 19 ± 4.4 yrs; mean disease duration 6 ± 3.2 yrs) were studied. Twenty healthy children and adolescents matched for social economical level were included as controls. Anti-HCV tests were performed using a third generation microparticle enzyme immunoassay. Inclusion criterion was low social economical level.
*Results:* The frequencies of anti-HCV antibody were low and comparable between JSLE and control group (2.5% vs. 0, $p = 1.0$). JSLE patients had significantly more risk factors for HCV infection compared to the control group, including immunosuppressive treatment (90% vs. 0, $p < 0.0001$), hospitalization (50% vs. 12.5%, $p = 0.0006$) and invasive procedures (47.5% vs. 12.5%, $p = 0.001$).
*Conclusions:* The observed low frequency of anti-HCV antibodies in high risk JSLE suggests that this virus does not seem to have a relevant role in the pathogenesis of this disease.


## Anticorpos contra o vírus da hepatite C em pacientes de alto risco com lúpus eritematoso sistêmico de início juvenil

*Palavras-chave:*
Vírus contra a hepatite C
Anti-VHC
Lúpus eritematoso sistêmico de início juvenil

RESUMO

*Objetivo:* Avaliar a prevalência de infecção pelo vírus da hepatite C (VHC) em pacientes de alto risco com lúpus eritematoso sistêmico de início juvenil (LESJ).
*Desenho do estudo:* Foram estudados 40 pacientes de baixa renda com LESJ (6H: 34M, com média de 19 ± 4,4 anos; duração média da doença de 6 ± 3,2 anos). Incluíram-se no grupo controle 20 crianças e adolescentes saudáveis pareados por nível socioeconômico. Fizeram-se testes anti-VHC com um ensaio imunoenzimático de micropartículas de terceira geração. O critério de inclusão foi o baixo nível socioeconômico.

* *Corresponding author.*
E-mail: nadia.aikawa@gmail.com (N.E. Aikawa).
http://dx.doi.org/10.1016/j.rbre.2016.02.011

*Resultados:* As frequências de anticorpos anti-VHC foram baixas e comparáveis entre os grupos LESJ e controle (2,5% versus 0, p = 1). Os pacientes com LESJ tinham significativamente mais fatores de risco para infecção por VHC em comparação com o grupo controle, incluindo tratamento imunossupressor (90% versus 0, p < 0,0001), internação (50% versus 12,5%, p = 0,0006) e procedimentos invasivos (47,5% versus 12,5%, p = 0,001).
*Conclusões:* A baixa frequência de anticorpos anti-VHC observada nos pacientes de alto risco com LESJ sugere que esse vírus não parece ter um papel relevante na patogênese dessa doença.



## Introduction

Systemic lupus erythematosus (SLE) is a complex autoimmune disease, characterized by a wide spectrum of multiorgan involvement and laboratorial abnormalities. The disease is characterized by several immunoregulatory alterations that culminate to an exacerbated production of autoantibodies. The pathogenesis of SLE is still unclear but some infectious agents, including hepatitis C virus (HCV), may be involved as possible triggering agents.[1,2]

HCV is a linear single stranded RNA virus of the *Flaviviridae* family, which has an extensive genomic variability.[3] It is well known that HCV infection is one of the most common causes of chronic viral hepatitis.[3] Furthermore, several extrahepatic immunological manifestations have been associated to HCV.[4–6]

In fact, HCV is an important inductor of autoantibody production such as antinuclear antibodies (ANA),[6] rheumatoid factors and cryoglobulins[7] and a possible association of this virus with autoimmune rheumatic diseases, including Sjögren's syndrome and vasculitis has been previously described.[8,9] In adult SLE patients some reports point out to a higher prevalence of anti-HCV antibodies (3–13%) than the expected for the general population[1,10,11] raising the possibility that exposure to this infection agent may contribute to disease expression.

There are, however, no data regarding the association of HCV in juvenile SLE (JSLE). Therefore, the aim of this study was to investigate the prevalence of sera anti-HCV antibodies in high risk JSLE patients.

## Patients and methods

Fifty consecutive low income subjects who fulfilled the American College of Rheumatology (ACR) classification criteria[12] for JSLE followed at our Pediatric Rheumatology Unit were initially selected. Ten of them were excluded due to incomplete charts and irregular follow-up. The remaining forty eligible patients were included in the study. Twenty healthy subjects matched by socioeconomic status (according to a standardized questionnaire of *Associação Brasileira dos Institutos de Pesquisa de Mercados*) were selected for the control group.[13]

High HCV risk factor was defined by low income [lowest Brazilian socio-economic classes (C, D or E)] and presence of one or more risk factors: hospitalizations, invasive diagnostic or therapeutic procedures (intravenous medications, biopsies or endoscopic diagnostic methods), immunosuppressive drugs, blood products transfusions, intravenous drugs use and promiscuous sexual activity (≥3 sexual partners per year).[14]

This study was approved by the Local Ethical Committee of our University Hospital.

For JSLE patients, clinical manifestations at disease diagnosis and at the moment of the study were searched. SLE disease activity at study entry was recorded for all patients using the SLE Disease Activity Index (SLEDAI) score[15] and an index greater than 8 was considered as active JSLE.

A systematic physical examination was performed to find signs of hepatic impairment, including cutaneous stigmata, liver enlargement, ascites and/or jaundice.

### *Detection of anti-HCV antibodies*

Testing for anti-HCV antibodies was performed in sera from all JSLE patients and controls using a third generation immunenzyme assay with MEIA – microparticle enzyme immunoassay (ELISA-3; Axsym System version 3.0; Abbott, Abbott Park, IL). The MEIA test is a variation of the standardized enzymatic immunoabsorbancy assay (EIA) developed to detect specific antibodies against structural and non-structural proteins of virus genome. This method uses plate containing recombinant HCV antigens Hcr43, c200, c100-3, pL33c and NS5.

### *Laboratory autoimmune and hepatic profile*

All subjects were screened for anti-nuclear antibodies (ANA) by indirect immunofluorescence using HEp-2 cells as substrate and anti-dsDNA antibody by indirect immunofluorescence using *Crithidia luciliae*. Further serologic evaluation included testing for Ro and La autoantibodies by countercurrent immunoelectrophoresis, anti-Sm and anti-ENA antibodies by hemmaglutination, IgM and IgG anti-cardiolipin by ELISA and rheumatoid factor by latex fixation and Waaler–Rose tests. In addition, protein electrophoresis, cryoglobulins (by cryoprecipitation) and total complement CH100 (by immunohemolysis) including C3 and C4 fractions (by radial immunodiffusion) were investigated.

Laboratory liver involvement was defined as the presence of elevated liver enzymes [aspartate aminotransferase (AST), alanino aminotransferase (ALT) and alkaline phosphatase] and bilirubin at least twice the normal values in two different occasions.

**Table 1 – Demographic data and risk factors for HCV infection in 40 JSLE patients and 20 controls.**

| | JSLE patient $n=40$ | Controls $n=20$ | $p$-Value |
|---|---|---|---|
| | $n$ (%) | | |
| *Demographic data* | | | |
| Female gender, $n$ (%) | 34 (85) | 16 (80) | 0.72 |
| Mean disease duration, years | 6 ± 3.2 | – | |
| Caucasian race, $n$ (%) | 36 (90) | 14 (70) | 0.07 |
| *Risk factor* | | | |
| Blood and blood components transfusion | 0 | 0 | 1.0 |
| Sexual promiscuity | 0 | 0 | 1.0 |
| Drug addiction | 0 | 0 | 1.0 |
| Low income | 40 (100) | 20 (100) | 1.0 |
| Immunosuppressive drug | 36 (90) | 0 | <0.0001 |
| Previous hospitalization | 20 (50) | 2 (10) | 0.004 |
| Number of invasive procedures | 19 (47.5) | 3 (15) | 0.022 |

JSLE, juvenile systemic lupus erythematosus; HCV, hepatitis C virus.

### *Statistical analysis*

Results are presented as the mean ± standard deviation for continuous variables and number (%) for categorical variables. Differences between categorical variables were assessed by Fisher's exact test. In all statistical tests significance was considered with a $p$ value < 0.05.

## Results

Thirty four (85%) JSLE patients and 16 (80%) control subjects were female with a mean age of 19 ± 4.4 years and 17 ± 3.9 years respectively ($p > 0.05$). The mean disease duration was 6 ± 3.2 years.

Frequencies of positive serum anti-HCV were low and comparable in JSLE and control group (2.5% vs. 0, $p = 1.0$). JSLE patients had significantly more risk factors for HCV infection compared to the control group, including immunosuppressive treatment (90% vs. 0, $p < 0.0001$), hospitalization (50% vs. 12.5%, $p = 0.0006$) and invasive procedures (47.5% vs. 12.5%, $p = 0.001$) (Table 1). None of them had history of contaminated blood product transfusion or sexual relation with a HCV individual.

All 47 JSLE patients' hospitalizations were due to either bacterial infections or to disease relapses and for the control group, the 5 hospitalizations were due to invasive procedures.

The only JSLE patient with detectable anti-HCV was a 23 year-old female patient with normal liver ultrasound in spite of slightly elevated transaminases levels (AST 61 IU/L, ALT 58 IU/L) and hypergammaglobulinemia. Her complement levels were normal, ANA was positive (1/200) and all other autoantibodies including antiliver antibodies and cryoglobulins were persistently negative. At the moment of the study, she had a mild malar rash, photosensitivity and arthritis with a SLEDAI <8 in spite of taking low dose GC and chloroquine.

The analysis of JSLE clinical manifestations demonstrated: 40 (100%) cutaneous and joints, 18 (46%) renal, 18 (46%) hematological and 15 (38%) neurological. More than one third of JSLE patients (15/39; 38%) had active disease (SLEDAI ≥8). Half of them were on high doses of glucocorticoid (GC), varying from 0.5 to 1.0 mg/kg/day, and 90% were under immunosuppressive treatment for a mean period of 1.5 ± 0.5 years [azathioprine in 22 (56%), methotrexate in 2 (5%) and cyclophosphamide in 12 (31%); 4 were on monthly GC pulse therapy].

Further laboratory analysis revealed that ten (26%) JSLE children had hypergammaglobulinemia and 12 (31%) had low complement levels compared to none in the control group ($p < 0.05$). Cryoglobulins were negative in sera from all JSLE and controls. ANA, anti-dsDNA, anti-Sm and anti-Ro were positive in sera from 100%, 50%, 27.5% and 17.5% of JSLE patients, respectively. Autoantibodies were uniformly negative in control group.

## Discussion

To our knowledge, this is the first study to demonstrate a low frequency of anti-HCV serology in a population of high risk JSLE patients from a tertiary University Hospital.

A limitation of the present study is that nowadays all adolescents and young adults are under risk of sexually transmitted diseases, including hepatitis C. This is a worldwide problem, and this issue is important not only to lower income families but to all families. Moreover, the low number of subjects in the control group herein hampers definitive conclusions regarding HCV in healthy subjects.

The prevalence of HCV infection among blood donors varies in different Brazilian geographic areas from 0.8% in smaller cities such as Curitiba, Paraná to 4.78% in our largest city São Paulo.[16,17] Data regarding HCV infection in healthy children and adolescents are however worldwide scarce. A study conducted by Martins[18] in Goiânia, Middle West of Brazil, revealed a prevalence of anti-HCV antibodies in this age bracket varying from 0.2 to 3%, similarly to children from a tertiary pediatric center in London, England (1.97%).[19]

The low frequency of these antibodies reported herein for JSLE was unexpected due to the fact that all patients

had low social levels and at least one additional known risk factor for HCV transmission such as immunosuppressive drugs use, hospitalizations and invasive interventions.[18,20] This low prevalence may be partially explained by the fact that patients were predominantly female and the virus seems to be influenced by gender being more frequent in men.[4] Accordingly, Karakoc et al. reported a frequency of 2.6% of anti-HCV by ELISA in a cohort of 38 SLE female patients, similar to the control population of the same region.[21] Moreover, in our Rheumatology outpatient clinic, JSLE patients are routinely oriented regarding sexual activity and protection against sexually transmitted diseases, therefore the low frequency of anti-HCV serology observed in the JSLE group can be related to a better education of the patients.

On the other hand, the methodology employed does not seem to account for the nearly absence of this reactivity in the present study since microparticles are used to increase the reactant surface allowing high levels of sensitivity and specificity.[22] In fact, none of our 15 patients with active disease (elevated SLEDAI index) revealed a positive test for anti-HCV in spite of previous report of false positive anti-HCV serology related to lupus activity.[5,11,23]

Additionally, HCV has also been associated to a high variety of extra hepatic manifestations including membranoproliferative glomerulonephritis, porphyria cutanea tarda, lychen planus and thyroiditis.[4–9] The notable capacity of HCV to induce autoantibodies has aroused particular interests in the possible association between SLE and HCV chronic hepatitis.[6] In fact, lupus patients concurrently infected by HCV were reported to express distinct features with lower frequency of cutaneous involvement and anti-dsDNA antibodies titers and higher frequency of liver injuries,[24] a pattern also observed in the only patient with this condition reported in the present study. Cryoglobulins are also frequently found in HCV positive contrasting to HCV-negative SLE patients,[25] although not observed in our patient.

In conclusion, this study suggests that HCV does not seem to represent an important complication in JSLE. The possible relevance of this virus in lupus manifestations remains to be determined.

## Conflicts of interest

The authors declare no conflicts of interest.

## REFERENCES


1. Ramos-Casals M, Font J, Garcia-Carrasco M, Cervera R, Jiménez S, Trejo O, et al. Hepatitis C virus infection mimicking systemic lupus erythematosus: study of hepatitis C virus infection in a series of 134 Spanish patients with systemic lupus erythematosus. Arthritis Rheum. 2000;43(12):2801–6.
2. Berkun Y, Zandman-Goddard G, Barzilai O, Boaz M, Sherer Y, Larida B, et al. Infectious antibodies in systemic lupus erythematosus patients. Lupus. 2009;18(13):1129–35.
3. Koziel MJ. Immunology of viral hepatitis. Am J Med. 1996;100(1):98–109.
4. Agmon-Levin N, Ram M, Barzilai O, Porat-Katz BS, Parikman R, Selmi C, et al. Prevalence of hepatitis C serum antibody in autoimmune diseases. J Autoimmun. 2009;32(3–4):261–6.
5. McMurray RW, Elbourne K. Hepatitis C virus infection and autoimmunity. Sem Arthritis Rheum. 1997;26(4):689–701.
6. Pawlotsky JM, Roudot-Thoraval F, Simmonds P, Mellor J, Ben Yahia MB, André C, et al. Extrahepatic immunologic manifestations in chronic hepatitis C virus serotypes. Ann Intern Med. 1995;122(3):169–73.
7. Lee YH, Ji JD, Yeon JE, Byun KS, Lee CH, Song GG. Cryoglobulinemia and rheumatic manifestations in patients with hepatitis C virus infection. Ann Rheum Dis. 1998;57(12):728–31.
8. Mariette X, Zerbib M, Jaccard A, Schenmetzler C, Danon F, Clauvel JP. Hepatitis C virus and Sjogren's syndrome. Arthritis Rheum. 1993;36(2):280–1.
9. Marcellin P, Descamps V, Martinot-Peignoux M, Larzul D, Xu L, Boyer N, et al. Cryoglobulinemia and vasculitis associated with hepatitis C virus infection. Gastroenterology. 1993;104(1):272–7.
10. Marchesoni A, Battafarano N, Podico M, Tosi S. Hepatitis C virus antibodies and systemic lupus erythematosus. Clin Exp Rheumatol. 1995;13(2):267–8.
11. Kowdley KV, Subler DE, Scheffel J, Moore B, Smith H. Hepatitis C virus antibodies in systemic lupus erythematosus. J Clin Gastroenterol. 1997;25(2):437–9.
12. Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum. 1997;40(9):1725.
13. Almeida PM, Wickerrhauser H. Critério de classe econômica da Associação Brasileira de Anunciantes (ABA) e Associação Brasileira dos Institutos de Pesquisa de Mercado (ABIPEME); 1991. p. 1–29.
14. Saraceno B, Kim JY. Alcohol use and sexual risk behaviour: a cross-cultural study in eight countries. World Health Organization; 2005. p. 54–6.
15. Bombardier C, Gladman DD, Urowitz MB, Caron D, Chang CH. Derivation of the SLEDAI. A disease activity index for lupus patients. Arthritis Rheum. 1992;35(6):630–40.
16. Parolin MB, Russo AA, de Almeida PT, Baldanzi GR, Lopes RW. Multicenter study on the prevalence of hepatitis C virus infection in blood donors in the city of Curitiba, Brazil. Arq Gastroenterol. 1999;36(3):117–21.
17. Ferraz ML, Adania RS, Figueiredo VW, Lanzoni VP, Silva AE. Significance of the presence of antibodies against hepatitis C virus in asymptomatic blood donors. Braz J Med Biol Res. 1996;29(10):1275–82.
18. Martins RM, Porto SO, Vanderborght BO, Rouzere CD, Queiroz DA, Cardoso DD, et al. Short report: prevalence of hepatitis C viral antibodies among Brazilian children, adolescents and street youths. Am J Trop Med Hyg. 1995;53(6):654–5.
19. McKeown C, Thaker U, Cubitt WD, Novelli V. Retrospective screening for hepatitis C in a tertiary paediatric referral centre. Commun Dis Public Health. 2003;6(1):40–3.
20. Alter MJ, Margolis HS, Krawczynski K, Judson FN, Mares A, Alexander WJ, et al. The natural history of community acquired hepatitis C virus in the United States. N Eng J Med. 1992;327(27):1899–905.
21. Karakoc Y, Dilek K, Gullulu M, Yavuz M, Ersoy A, Akalyn H, et al. Prevalence of hepatitis C virus antibody in patients with systemic lupus erythematosus. Ann Rheum Dis. 1997;56(9):570–1.
22. Kuo G, Choo QL, Alter HJ, Gitnick GL, Redeker AG, Purcell RH, et al. An assay for circulating antibodies to a major etiologic virus of human non-A, non-B hepatitis. Science. 1989;244(4902):362–4.

23. Barron KS, Silverman ED, Gonzales J, Reveille JD. Clinical, serologic and immunogenetic studies in childhood-onset systemic lupus erythematosus. Arthritis Rheum. 1993;36(3):348–54.
24. Feng H, Zhang GY, Xie HF, Chen ML, Shi W, Wang LY. Hepatitis C virus infection combined with systemic lupus erythematosus. Zhong Nan Da Xue Xue Bao Yi Xue Ban. 2006;31(6):891–3.
25. Perlemuter G, Cacoub P, Sbaï A, Hausfater P, Thibault V, Le TH, et al. Hepatitis C virus infection in systemic lupus erythematosus: a case–control study. J Rheumatol. 2003;30(7):1473–8.