Organ-specific autoantibodies and autoimmune diseases in juvenile systemic lupus erythematosus and juvenile dermatomyositis patients

N.E. Aikawa[1,2], A.A. Jesus[1], B.L. Liphaus[1], C.A. Silva[1,2], M. Carneiro-Sampaio[3], V.S.T. Viana[2], A.M.E. Sallum[1]

[1]Paediatric Rheumatology Unit, [2]Rheumatology Division and [3]Allergy and Immunology Unit of Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil.

Abstract

Objectives

To our knowledge, no study assessed simultaneously a variety of organ-specific autoantibodies and the prevalence of organ-specific autoimmune diseases in juvenile systemic lupus erythematosus (JSLE) and juvenile dermatomyositis (JDM). Therefore, the purpose of this study was to evaluate organ-specific autoantibodies and autoimmune diseases in JSLE and JDM patients.

Methods

Forty-one JSLE and 41 JDM patients were investigated for autoantibodies associated with autoimmune hepatitis, primary biliary cirrhosis, type 1 diabetes mellitus (T1DM), autoimmune thyroiditis (AT), autoimmune gastritis and coeliac disease (CD). Patients with positive antibodies were investigated for the respective organ-specific autoimmune diseases.

Results

Mean age at diagnosis was higher in JSLE compared to JDM patients (10.3±3.4 vs. 7.3±3.1years, p=0.0001). The frequencies of organ-specific autoantibodies were similar in JSLE and JDM patients (p>0.05). Of note, a high prevalence of T1DM and AT autoantibodies was observed in both groups (20% vs. 15%, p=0.77 and 24% vs. 15%, p=0.41; respectively). Higher frequencies of ANA (93% vs. 59%, p=0.0006), anti-dsDNA (61% vs. 2%, p<0.0001), anti-Ro, anti-Sm, anti-RNP, anti-La and IgG-aCL were observed in JSLE (p<0.05). Organ-specific autoimmune diseases were evidenced only in JSLE patients (24% vs. 0%, p=0.13). Two JSLE patients had T1DM associated with Hashimoto thyroiditis and another had subclinical thyroiditis. Another JSLE patient had CD diagnosis based on iron deficiency anaemia, anti-endomysial antibody, duodenal biopsy compatible to CD and response to a gluten-free diet.

Conclusions

Organ-specific diseases were observed solely in JSLE patients and required specific therapy. The presence of these antibodies recommends the evaluation of organ-specific diseases and a rigorous follow-up.

Key words

organ-specific, autoantibodies, juvenile systemic lupus erythematosus, juvenile dermatomyositis

Nádia E. Aikawa, MD
Adriana A. Jesus, MD
Bernadete L. Liphaus, MD, PhD
Clovis A. Silva, MD, PhD
Magda Carneiro-Sampaio, MD, PhD
Vilma S.T. Viana, PhD
Adriana M.E. Sallum, MD, PhD

Please address correspondence to:
Dr Nádia Emi Aikawa,
Rua Oscar Freire 1456, Apto 53,
Jardim América,
São Paulo - SP, Brazil.
E-mail: nadia.aikawa@gmail.com

Received on March 19, 2011; accepted in revised form on June 21, 2011.

Funding: This study was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo – FAPESP (grant no. 08/58238-4 to M. Carneiro-Sampaio), by Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq (grant no. 300248/2008-3 to C.A. Silva) and Federico Foundation Grant to C.A. Silva.
Competing interests: none declared.

Introduction

Studies in juvenile systemic lupus erythematosus (JSLE) patients have shown high prevalences of anti-thyroid antibodies (1). Additionally, autoimmune hepatitis was rarely reported in our JSLE patients (2). However, few studies described organ-specific antibodies in juvenile dermatomyositis (JDM) (3-6). To our knowledge, other organ-specific autoimmune diseases, such as coeliac disease (CD), autoimmune gastritis and primary biliary cirrhosis, were not evaluated in both diseases. Moreover, no study assessed simultaneously a large number of organ-specific autoantibodies and subclinical organ-specific autoimmune diseases in JSLE and JDM patients. Therefore, this was a cross-sectional study that investigated the organ-specific and other serum autoantibodies in our JSLE and JDM populations and described the associated organ-specific diseases.

Patients and methods

From January 2008 to January 2009, 79 JSLE and 52 JDM patients were followed at the Paediatric Rheumatology Unit of our University Hospital. Of them, 41 JSLE and 41 JDM patients agreed to participate in this study. All patients fulfilled the American College of Rheumatology (ACR) criteria for JSLE (7) and the Bohan and Peter criteria for JDM (8). No patient had JSLE and JDM overlap syndrome. The local ethics committee approved this study and an informed consent was obtained from all participants.

Organ-specific and other autoantibodies

Serum autoantibodies associated with the following organ-specific autoimmune diseases were assessed at study entry:

Autoimmune thyroiditis (AT): anti-thyroid peroxidase (anti-TPO) antibody using kits from Wallac AutoDELPHIA indirect fluoroimmunoassay (manufacturer's recommended reference value <35 IU/mL), anti-thyroglobulin (anti-TG) antibody (Wallac AutoDELPHIA indirect fluoroimmunoassay; reference value <35 IU/mL) and anti-thyroid stimulating hormone (TSH) receptor antibody (TRAb) by radio receptor assay (TSH receptor antibody assay, Kronus, Boise, ID, USA; reference value <8%); *T1DM* - insulin autoantibody (IAA) (RSR Ltd, Cardiff, UK; reference value <0.4 IU/mL), anti-glutamic acid decarboxylase (anti-GAD) (RSR Ltd, Cardiff, UK; reference value <25 IU/mL) and anti-tyrosine phosphatase (anti-IA2) antibody by radioimmunoassay (RSR Ltd, Cardiff, UK; reference value <125 IU/mL);

Autoimmune hepatitis: anti-type I liver-kidney microsomal (anti-LKM-1) antibody on frozen sections of rodent kidney, liver and stomach tissue substrates (Dako, Copenhagen, Denmark; reference value <1:20) and anti-smooth muscle antibody (SMA) by indirect immunofluorescence on rat liver and kidney tissue sections on frozen sections of rodent kidney, liver and stomach tissue substrates (Dako, Copenhagen, Denmark; reference value <1:20);

Primary biliary cirrhosis: antimitochondrial antibody (AMA) by indirect immunofluorescence on rat liver, kidney and stomach parietal cells (Dako, Copenhagen, Denmark; reference value <1:10), and confirmation of the positive cases by enzyme-linked immunosorbent assay (ELISA);

Autoimmune gastritis: parietal cell autoantibody (PCA) by indirect immunofluorescence cells using rat stomach sections as substrate (Dako, Copenhagen, Denmark; reference value <1:10);

Coeliac disease (CD): immunoglobulin A (IgA) class anti-endomysial (EMA) antibody of IgA isotype by indirect immunofluorescence using umbilical cord as substrate (Dako, Copenhagen, Denmark; reference value <1:10). Patients who were positive for organ-specific autoantibodies had the test repeated for confirmation after a median time of 6 (4-9) months in JDM and 8 (4-12) months in JSLE patients. At the moment of the study, they were also investigated for the presence of the organ-specific autoimmune disease.

The following other serum autoantibodies were also measured at study entry: antinuclear antibody (ANA) by indirect immunofluorescence on human cell epithelioma (HEp-2) cells (Euroimmun AG, Germany) and staining reactiv-

ity at ≥1:80 serum dilution defined as positive; rheumatoid factor (RF) by immunonephelometry (cut-off value of 15 IU/mL); anti-double-stranded DNA (anti-dsDNA) by in-house indirect immunofluorescence using *Crithidia luciliae* as substrate (cut-off value of 1:10); anti-Sm and anti-RNP by in-house passive haemagglutination using chicken erythrocytes sensitised with rabbit thymus extract as the source of antigen (cut-off of 1:100); anti-SSA/Ro and anti-SSB/La by in-house counterimmunoelectrophoresis against dog spleen saline extract (reference: negative); anti-topoisomerase 1 (anti-Scl70) by ELISA (ELISA Hemagen Diagnostics Inc., Columbia, NY; reference value ≤10 IU/mL), anticardiolipin (aCL) isotypes IgG and IgM by ELISA (QUANTA Lite, INOVA, USA; cut-off value of 20 GPL and/or MPL). Lupus anticoagulant (LAC) by the dilute Russell's viper venom time with confirmatory testing. Anti-neutrophil cytoplasm antibody (ANCA) was detected by indirect immunofluorescence assay using ethanol-fixed human neutrophils isolated from peripheral blood from healthy volunteers and fluorescein-labeled goat anti-human IgG (Sigma Chemical Co., St Louis, MO; reference value <1:20). Moreover, the following myositis-specific and myositis-associated antibodies were determined: anti-Jo1, anti-Mi2, anti-PL-7, anti-PL-12, anti-Ku and anti-PM-Scl, using a commercial available line blot immunoassay (EUROLINE Myositis profile, EUROIMMUN AG, Lüebeck, Germany). Serum testing (1:101 diluted) was performed according manufacturer's protocol and results were kindly scanned and evaluated with the computer program EUROLineScan by EUROIMMUN AG.

Organ-specific autoimmune diseases

HT was defined as reduced free thyroxine (T4) and elevated TSH levels, and subclinical hypothyroidism as elevated TSH associated with normal T4 (9). The presence of antithyroid antibodies was required to characterise AT. T1DM was diagnosed by polyuria, polydipsia and unexplained weight loss, and increased plasma glucose ≥200 mg/dL at any time of day or fasting glucose ≥126 mg/dL (10). Autoimmune hepatitis was defined as a progressive chronic hepatitis of unknown origin, with elevated transaminase levels, hypergammaglobulinemia, serum autoantibodies and histological characteristics (2). Primary biliary cirrhosis was defined as at least two of the following: elevated alkaline phosphatase (≥2 times the upper limit of normal) or gamma-glutamiltrasferase (≥5 times the upper limit of normal), positivity for AMA, and liver biopsy with nonsuppurative cholangitis and destruction of bile ducts (11). Autoimmune gastritis was defined by gastric atrophy in histology, PCA and anti-intrinsic factor positivity, hypo/achlorhydria, low concentrations of serum pepsinogen and anaemia secondary to vitamin B12 and iron deficiency (12). CD was defined by at least four of the following: clinical manifestations (chronic diarrhoea, stunting and/or iron deficiency anaemia), positivity for CD IgA antibodies, HLA-DQ2 or DQ8 genotype, small intestine biopsy compatible with coeliac enteropathy, and response to gluten-free diet (13).

Disease activity, disease damage and treatment in JSLE and JDM patients

SLE disease activity and cumulative damage were measured at the moment of organ-specific antibodies and disease evaluations in JSLE patients using the SLE Disease Activity Index 2000 (SLEDAI-2K) (14) (range 0–105) and the Systemic Lupus International Collaborating Clinics/ACR Damage Index (SLICC/ACR-DI) (15) (range 0–42).

JDM activity was assessed by disease activity score (DAS) (16) (range 0–20), and muscle strength was evaluated by childhood myositis assessment scale (CMAS) (17) (range 0–51) and manual muscle testing (MMT) (18) (range 0–80). The serum muscle enzymes performed were creatine phosphokinase (CPK) (normal range 39–170 UI/L), aspartate aminotransferase (AST) (normal range 10–36 UI/L), alanine aminotransferase (ALT) (normal range 24-49 UI/L), lactate dehydrogenase (LDH) (normal range 240-480 UI/L) and aldolase (normal range <7.6UI/L).

Data concerning the current JSLE and JDM treatments included: prednisone, methotrexate, azathioprine, chloroquine, cyclosporine, cyclophosphamide, mycophenolate mofetil and intravenous immunoglobulin.

Statistical analysis

The results were presented as mean ± standard deviation or median (range) for continuous and number (%) for categorical variables. Data were compared by Student's *t*- or Mann-Whitney tests for continuous variables. For categorical variables differences were assessed by Fisher's exact test. In all the statistical tests the level of significance was set at 5% ($p<0.05$).

Results

Demographic features

The mean age at JSLE diagnosis was significantly higher compared to JDM patients (10.3±3.4 *vs*. 7.3±3.1 years, $p=0.0001$). However, the mean duration of disease was similar in both groups (4.4±3.7 *vs*. 4.4±3.3 years, $p=0.92$), as well as the frequency of female gender (85% *vs*. 71%, $p=0.18$) (Table I).

Organ-specific and other autoantibodies

The organ-specific autoantibodies were assessed at the time of disease diagnosis in one JSLE and in four JDM patients and during disease flare in 14 JSLE and in 16 JDM patients.

The frequencies of at least one serum organ-specific antibody were similar in JSLE and JDM patients [17 (41%) *vs*. 11 (27%), $p=0.24$]. High frequencies of AT autoantibodies were observed in both diseases (24% *vs*. 15%, $p=0.41$; 20% *vs*. 15%, $p=0.77$; respectively). The frequencies of EMA, PCA, anti-LKM-1 antibody and/or SMA were comparable in both groups (Table I).

Higher frequencies of ANA (93% *vs*. 59%, $p=0.0006$), anti-dsDNA (61% *vs*. 2%, $p<0.0001$), anti-Ro (35% *vs*. 0%, $p<0.0001$), anti-Sm ($p=0.01$), anti-RNP ($p=0.02$), anti-La ($p=0.03$) and IgG aCL ($p=0.001$) were observed in JSLE compared to JDM patients. The frequencies of myositis-specific and myositis-associated antibodies were similar in JSLE and JDM patients ($p>0.05$) (Table I).

The median of current age and disease duration, and the frequencies of

Table I. Demographic data, organ-specific autoantibodies and diseases, and other autoantibodies in juvenile systemic lupus erythematosus (JSLE) versus juvenile dermatomyositis (JDM) patients.

Variables	JSLE n = 41	JDM n = 41	*p*-value
Demographic data			
Current age, years	14.3 ± 3.8	11.7 ± 4.6	0.007
Disease duration, years	4.4 ± 3.7	4.4 ± 3.3	0.92
Female gender	35 (85)	29 (71)	0.18
Organ-specific antibodies	17 (41)	11 (27)	0.24
Autoimmune thyroiditis (anti-TG and/or anti-TPO and/or TRAb)	10 (24)	6 (15)	0.41
Type 1 diabetes mellitus (IAA and/or anti-GAD and/or anti-IA2)	8 (20)	6 (15)	0.77
Coeliac disease (EMA)	1 (2)	1 (2)	1.0
Autoimmune hepatitis (SMA and/or anti-LKM-1)	1 (2)	2 (5)	1.0
Primary biliar cirrhosis (AMA)	0 (0)	0 (0)	1.0
Autoimune gastritis (PCA)	1 (2)	0 (0)	1.0
Organ-specific diseases	4/17 (24)	0/11 (0)	0.13
Other autoantibodies			
ANA	38 (93)	24 (59)	0.0006
RF	4 (10)	0 (0)	0.12
Anti-dsDNA	25 (61)	1 (2)	<0.0001
Anti-Sm	11 (27)	2 (5)	0.01
Anti-RNP	9 (22)	1 (2)	0.02
Anti-Ro	14 (35)	0 (0)	<0.0001
Anti-La	6 (15)	0 (0)	0.03
Anti-Scl-70	0 (0)	0 (0)	1.0
aCL-IgM	20 (49)	17 (41)	0.66
aCL-IgG	19 (46)	5 (12)	0.001
LAC	5 (12)	1 (2)	0.2
p-ANCA	4 (10)	0 (0)	0.12
c-ANCA	1 (2)	1 (2)	1.0
Anti-Jo1	0/6 (0)	0/38 (0)	1.0
Anti-Mi2	1/6 (17)	1/38 (3)	0.26
Anti-PL-7	0/6 (0)	0/38 (0)	1.0
Anti-PL-12	0/6 (0)	0/38 (0)	1.0
Anti-Ku	2/6 (33)	5/38 (13)	0.24
Anti-PM-Scl	0/6 (0)	0/38 (0)	1.0

Data are expressed in n (%); anti-TG: anti-thyroglobulin antibody; anti-TPO: anti-thyroid peroxidase antibody; TRAb: anti-thyroid stimulating hormone (TSH) receptor antibody; IAA: insulin autoantibody; anti-GAD: anti-glutamic acid decarboxylase antibody; anti-IA2: anti-tyrosine phosphatase antibody; EMA: anti-endomysial antibody; SMA: anti-smooth muscle antibody; anti-LKM-1: anti- type I liver-kidney microsomal antibody; AMA: antimitochondrial antibody; PCA: parietal cell autoantibody; ANA: antinuclear antibody; RF: rheumatoid factor; anti-dsDNA: anti-double-stranded DNA; anti-Scl70: anti-topoisomerase 1; aCL: anticardiolipin; LAC: lupus anticoagulant; p-ANCA: perinuclear anti-neutrophil cytoplasm antibody; c-ANCA: cytoplasmic anti-neutrophil cytoplasm antibody; anti-Jo1: anti-hystidil-tRNA synthetase; anti-Mi2: anti-nuclear ATPase/helicase; anti-PL-7: anti-threonyl-tRNA synthetase; anti-PL-12: anti-alanyl-tRNA synthetase; anti-Ku: anti-Ku-p350 complex.

female gender and current treatment (prednisone and immunosuppressive use) were alike in JSLE patients with (n=17) *versus* without (n=24) organ-specific antibody ($p>0.05$). The median of SLEDAI-2K and SLICC/ACR-DI were similar in both groups [5 (0–12) *vs*. 3 (0–14), $p=0.36$; 1 (0–2) *vs*. 1 (0–2), $p=0.93$; respectively]. Likewise, no differences were evidenced in the median of current age, disease duration and muscle enzyme levels (AST, ALT, CPK, aldolase and LDH), and the frequencies of female gender and current treatment (prednisone and immunosuppressive use) in JDM patients with (n=11) *versus* without (n=30) organ-specific antibody ($p>0.05$). The median of CMAS, DAS and MMT were similar in both groups [50 (44–52) *vs*. 48.5 (4–52), $p=0.27$; 80 (75–80) *vs*. 80 (38–80), $p=0.47$; 2 (0–7) *vs*. 3 (0–17), $p=0.71$; respectively].

No differences were observed in demographic data, disease activity, treatment and other autoantibodies frequencies in 17 JSLE patients with at least one organ-specific autoantibody compared to 24 without these autoantibodies ($p>0.05$). Additionally, no differences were evidenced in these parameters in 11 JDM patients with at least one organ-specific autoantibody *versus* 30 without these autoantibodies ($p>0.05$).

Organ-specific autoimmune diseases

No patients had assessment of organ-specific autoantibodies preceding the onset of an organ-specific disease. Organ-specific autoimmune diseases were evidenced only in JSLE patients (24% *vs*. 0%, $p=0.13$) (Table I). Two of them fulfilled both T1DM and HT diagnosis criteria and were treated with insulin and levothyroxine. Another patient had subclinical hypothyroidism with presence of anti-TG antibody. The fourth patient had diagnosis of CD based on the following features: chronic iron deficiency anaemia, presence of AEM antibody, duodenal biopsy compatible to CD and response to a gluten-free diet (Table II).

None of our 41 JDM patients had evidence of organ-specific autoimmune diseases.

Discussion

As far as we know, this was the first study to evaluate simultaneously a variety of organ-specific antibodies in JSLE and JDM, and demonstrated a high prevalence of these antibodies in both diseases. Organ-specific autoimmune diseases were evidenced exclusively in JSLE, particularly autoimmune endocrine and gastrointestinal illnesses that required specific treatment.

Of note, JSLE is a chronic multisystem autoimmune disease, with a marked risk for the development of multiple autoantibodies (1). The profile of other specific and non-specific antibodies was clearly evidenced in our JSLE patients compared to JDM. The main limitation of the present study was the lack of comparison with a control population of healthy children and adolescents.

Clinical AT, especially HT, is the most important organ-specific autoimmune

Table II. Demographic data, disease activity, other autoantibodies and treatment in four juvenile systemic lupus erythematosus (JSLE) patients with organ-specific autoimmune diseases.

Case	1	2	3	4
Demographic data				
Age at JSLE diagnosis, years	11.6	15.6	11.1	9.3
Age at organ-specific autoimmune disease, years	11.4	12	11.6	13.2
Current age, years	15	18.9	16.3	12.6
Gender	female	female	female	female
Organ-specific autoimmune disease	T1DM and HT	T1DM and HT	Subclinical hypothyroidism	Coeliac disease
Organ-specific antibodies	Anti-TPO and IAA	TRAb, IAA and Anti-GAD	Anti-TG	EMA
Clinical features at JSLE diagnosis				
Mucocutaneous involvement	–	+	–	+
Arthritis	+	–	+	–
Serositis	+	–	+	+
Haematological abnormalities	–	+	+	–
Neuropsychiatric involvement	–	–	–	–
Nephritis	–	+	+	–
Disease activity and damage at organ-specific antibodies assessment				
SLEDAI-2K	8	8	12	8
SLICC/ACR-DI	2	1	1	0
Current treatment				
JSLE	Prednisone	Prednisone, chloroquine and azathioprine	Prednisone, chloroquine and azathioprine	Prednisone and chloroquine
Organ-specific autoimmune disease	Insulin and levothyroxine supplementation	Insulin and levothyroxine supplementation	–	Gluten-free diet

T1DM: type 1 diabetes mellitus; HT: Hashimoto thyroiditis; Anti-TPO: anti-thyroid peroxidase antibody; IAA: insulin autoantibody; TRAb: anti-thyroid stimulating hormone (TSH) receptor antibody; Anti-GAD: anti-glutamic acid decarboxylase antibody; Anti-TG: anti-thyroglobulin antibody; EMA: anti-endomysial antibody; SLEDAI-2K: Systemic Lupus Erythematosus Disease Activity Index 2000; SLICC/ACR-DI: Systemic Lupus International Collaborating Clinics/ACR (SLICC/ACR) Damage Index; + positive, - negative

disease in female JSLE. The frequency of anti-thyroid antibodies was previously reported in 14–26% and autoimmune subclinical hypothyroidism in 0–7% of JSLE population, as also observed in the current study (1, 19).

Interestingly, no study evaluated the frequency of T1DM-associated antibodies in JSLE population, and few reports described pancreas autoantibodies in adult and paediatric lupus (6). In our population, we found 5% of JSLE patients with these antibodies and controlled insulin-dependent T1DM.

Another relevant aspect of our study was the assessment of gastrointestinal autoimmunity. One of our JSLE patients with chronic iron deficient anaemia and without clinical manifestations had CD. Indeed, the most common manifestations of CD are weight loss and diarrhea, and anaemia is found in the subclinical forms (13).

PCA antibody is highly correlated with chronic autoimmune gastritis (12). The absence of gastrointestinal manifestations may indicate that the autoimmune process was at an initial stage in one of our JSLE patients with PCA. Importantly, chronic autoimmune gastritis can progress within up to 30 years period (12), and this patient requires a rigorous follow-up.

Furthermore, previous studies with small JDM populations and incomplete evaluations reported assessment of organ-specific antibodies (3, 4). We observed only endocrine, liver and intestinal autoantibodies in JDM without organ-specific autoimmune diseases, as previously described (4). Montecucco *et al*. (3) did not evidence autoimmune liver and gastric autoantibodies in 14 JDM patients, and Martinez-Cordero *et al*. (4) described one JDM patient with SMA.

The myositis-specific and myositis-associated antibodies were demonstrated in both diseases, and to our knowledge, these autoantibodies were not studied in paediatric lupus.

Disease activity and treatment were not associated with organ-specific autoantibodies in our two autoimmune disease populations. In contrast, a high frequency of anti-thyroid antibodies and subclinical thyroiditis were previously evidenced in mild JSLE patients (1). Moreover, fluctuation of these antibodies may occur during the course of the disease, as described in JSLE with autoimmune thyroid disease (1, 9).

This was a cross-sectional study and organ-specific diseases, such as CD and AT, were diagnosed at the moment of autoantibodies evaluation. Of note, all patients are followed in our Outpatient Paediatric Rheumatology Unit. JSLE patients should be annually screened for the presence of organ-specific antibodies related to AT and DM1. Furthermore, those with chronic anaemia should be investigated with specific IgA class antibodies for CD, and upper gastrointestinal endoscopy with duodenal biopsy is required in patients with this autoantibodies.

In conclusion, organ-specific diseases

were observed solely in JSLE patients and required specific treatment. The presence of these antibodies recommends the evaluation of organ-specific diseases and a rigorous follow-up of these patients.

Acknowledgements

We thank Prof. Suely Marie for the technical support and Prof. Leila Antonângelo of the Central Laboratory Division of our Hospital.

References

1. PARENTE COSTA L, BONFÁ E, MARTINAGO CD, DE OLIVEIRA RM, CARVALHO JF, PEREIRA RM: Juvenile onset Systemic Lupus Erythematosus thyroid dysfunction: a subgroup with mild disease? *J Autoimmun* 2009; 33: 121-4.
2. DEEN ME, PORTA G, FIOROT FJ, CAMPOS LM, SALLUM AM, SILVA CA: Autoimmune hepatitis and juvenile systemic lupus erythematosus. *Lupus* 2009; 18: 747-51.
3. MONTECUCCO C, RAVELLI A, CAPORALI R *et al*.: Autoantibodies in juvenile dermatomyositis. *Clin Exp Rheumatol* 1990; 8: 193-6.
4. MARTÍNEZ-CORDERO E, MARTÍNEZ-MIRANDA E, AGUILAR LEÓN DE: Autoantibodies in juvenile dermatomyositis. *Clin Rheumatol* 1993; 12: 426-8.
5. GO T, MITSUYOSHI I: Juvenile dermatomyositis associated with subclinical hypothyroidism due to auto-immune thyroiditis. *Eur J Pediatr* 2002; 161: 358-9.
6. LIDAR M, BRAF A, GIVOL N *et al*.: Anti-insulin antibodies and the natural autoimmune response in systemic lupus erythematosus. *Lupus* 2001; 10: 81-6.
7. HOCHBERG MC: Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 1997; 40: 1725.
8. BOHAN A, PETER JB: Polymiositis and dermatomyositis. *N Engl J Med* 1975; 13: 344-7.
9. FRANKLYN JA: Hypothyroidism. *Medicine* 2005; 33: 27-9.
10. AMERICAN DIABETES ASSOCIATION: Standards of medical care in diabetes 2007 (Positional Statement). *Diabetes Care* 2007; 30: S4-S41.
11. KAPLAN MM, GERSHWIN ME: Primary biliary cirrhosis. *N Engl J Med* 2005; 353: 1261-73.
12. DE BLOCK CE, DE LEEUW IH, VAN GAAL LF: Autoimmune gastritis in type 1 diabetes: a clinically oriented review. *J Clin Endocrinol Metab* 2008; 93: 363-71.
13. CATASSI C, FASANO A: Celiac disease diagnosis: simple rules are better than complicated algorithms. *Am J Med* 2010; 123: 691-3.
14. GLADMAN DD, IBANEZ D, UROWITZ MB: Systemic lupus erythematosus disease activity index 2000. *J Rheumatol* 2002; 29: 288-91.
15. BRUNNER HI, SILVERMAN ED, TO T, BOMBARDIER C, FELDMAN BM: Risk factors for damage in childhood-onset systemic lupus erythematosus: cumulative disease activity and medication use predict disease damage. *Arthritis Rheum* 2002; 46: 436-44.
16. BODE RK, KLEIN-GITELMAN MS, MILLER ML, LECHMAN TS, PACHMAN LM: Disease activity score for children with juvenile dermatomyositis: reliability and validity evidence. *Arthritis Rheum* 2003; 49: 7-15.
17. LOVELL DJ, LINDSLEY CB, RENNEBOHM RM *et al*.: Development of validated disease activity and damage indices for the juvenile idiopathic inflammatory myopathies. II. The Childhood Myositis Assessment Scale (CMAS): a quantitative tool for the evaluation of muscle function. The Juvenile Dermatomyositis Disease Activity Collaborative Study Group. *Arthritis Rheum* 1999; 42: 2213-9.
18. RIDER LG, KOZIOL D, GIANNINI EH *et al*.: Validation of manual muscle testing and a subset of eight muscles for adult and juvenile idiopathic inflammatory myopathies. *Arthritis Care Res* 2010; 62: 465-72.
19. RONCHEZEL MV, LEN CA, SPINOLA E CASTRO A *et al*.: Thyroid function and serum prolactin levels in patients with juvenile systemic lupus erythematosus. *J Pediatr Endocrinol Metab* 2001; 14: 165-9.