Molecular mimicry has been proposed as a pathogenic mechanism for autoimmune disease. The hypothesis is based on the epidemiological, clinical, and experimental studies and in evidence finding an association between infectious agents and autoimmune disease, observing cross-reactivity of immune agents with host antigens and microbial determinants.1

Recent studies have revealed that carbohydrate mimicry of bacterial lipo-oligosaccharide by the human ganglioside is an important cause of Guillain-Barré syndrome, for example. This new concept that carbohydrate mimicry can cause an autoimmune disease provides a clue of the pathogenesis of immune-mediated diseases.1

Molecular mimicry is based on a structural similarity between a pathogen or metabolite and self-structure. The similarity could be expressed as shared amino acid sequences or as a similar conformational structure between a pathogen and self-antigen.2 The strongest association of viruses and type 1 diabetes (T1D) involves enterovirus species, of which some strains have the ability to induce or accelerate autoimmune disease in animal models.

Several hypotheses regarding the mechanism to explain how viruses affect islet autoimmunity and beta-cell destruction were proposed.3 Viral infection may serve as an accelerating factor that, superimposed onto advanced insulinitis, leads to rapid culmination into hyperglycemia. Rubella virus is a possible environmental agent which may be involved in the triggering of autoimmunity to pancreatic islet cells, leading to T1D. Autoantibody responses were found in 239 10-year-old girls who received live attenuated rubella vaccine, of whom 61 (26%) had no pre-existing rubella immunity.4 Infection can promote the expression of human endogenous retroviruses by molecular mimicry or by functional mimicry.5 There are additional mechanisms which may control the expression of human endogenous retroviruses, such as the epigenetic status of the genome.6

T1D develops during months to years, in which islet autoimmunity destroys the insulin-producing beta cells of the pancreas. This period is marked by the presence of antibodies to insulin, glutamic acid decarboxylase, and tyrosine phosphatase IA-2, as well as by islet-reactive T cells, but how islet autoimmunity is initiated and accelerated is not well defined. The incidence of T1D in many countries has risen rapidly over the past 30–50 years, mainly among the young population. The hypothesis explains that it is an increased environmental pressure on susceptibility genotypes.

A rapid rise in the prevalence of virus infections could perhaps explain this phenomenon by increasing the frequency of diabetogenic infections. The following criteria have been proposed for disease causality:

- Temporal relationship: exposure precedes disease – the only absolutely essential criterion.
- Statistical strength of association: the higher the more convincing.
- Dose-response relationship: increasing exposure increases risk.
- Consistency: replication of results by different methods or in different populations.
- Plausibility: how well the data agree with current concepts of pathological mechanisms.
- Consideration and/or rejection of other alternative explanations.
- Experiments: can the findings be replicated experimentally?
- Specificity: the weakest criterion. Lack of dose specificity does not negate causality, but, if present, strengthens the claim.
- Coherence: is the association compatible with the existing body of knowledge?

Rubella fulfills the criteria for causality of T1D, with statistically strong temporal association that is consistent, plausible, and specific. Despite strong data and the association between T1D-susceptibility and HLA the mechanism of rubella-associated T1D remains unresolved and rubella vaccination is clearly not the answer to prevention of T1D.7

Rubella virus infection during pregnancy is known to spread to the fetus in the majority of seronegative mothers. If the infection occurs during the first trimester of pregnancy, there is high risk of serious organ damage in the fetus.
A variety of clinical abnormalities is seen in congenital rubella syndrome, including endocrine diseases such as Addison’s disease, growth hormone deficiency, and increased frequency of diabetes. In the case of viral triggering of autoimmune T1D, certain viruses (retrovirus in NOD mice, rubella virus in hamsters and humans) may alter a normally existing beta cell antigen into immunogenic form or might induce a new antigen, leading to beta cell-specific autoimmune insulin dependent diabetes mellitus. In addition, other viruses could generate antigen-specific T effectors cells which may cross-react with a beta cell-specific autoantigen.

Another issue raised up in the last years is whether vaccination can induce autoimmunity. Autoimmune reactions to vaccinations may rarely be induced in predisposed individuals by molecular mimicry or bystander activation mechanisms. Vaccine-associated autoimmune reactions include Guillain-Barré syndrome after 1976 swine influenza vaccine and immune thrombocytopenic purpura after measles/mumps/ rubella vaccine.

Vaccines can cause adverse events, and most of the side effects are mild and transient; however, reactions such as hypersensitivity, induction of infection, and autoimmunity do occur. The rarity and subacute presentation of post-vaccination autoimmune phenomena means that ascertaining causality between these events can be difficult. Moreover, the latency period between vaccination and autoimmunity ranges from days to years.

Experimental animal model, in which the causal relation between vaccines and autoimmune findings has been examined: in healthy puppies immunized with commonly given vaccines, a variety of autoantibodies have been documented, but no autoimmune illness was found. The findings could also represent a polyclonal activation (adjuvant reaction).

The mechanisms of autoimmune reactions following immunization have not been elucidated. As mentioned above, one of the possibilities is molecular mimicry, when a structural similarity exists between some viral antigen and self-antigen. Various environmental factors in the pathogenesis of immune mediated diseases are well established, of which factors entailing an immune adjuvant activity, such as infectious agents, silicone, aluminum salts, and others were associated with defined and non-defined immune mediated diseases, both in animal models and in humans. In recent years a syndrome entitled ASIA (Autoimmune Syndrome Induced by Adjuvants), comprising four conditions (siliconosis, the Gulf war syndrome, macrophagic myofasciitis syndrome, and post-vaccination phenomena), has been linked with previous exposure to an adjuvant. Further epidemiological studies are needed to obtain more data for the issues raised above.

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EDITORIAL

Sabe-se que a infecção pelo vírus da rubéola durante a gravidez atinge o fetó na maioria das mães soronegativas. Quando a infecção ocorre no primeiro trimestre gestacional, o risco de graves lesões em órgãos fetais é alto. Uma grande variedade de anormalidades clínicas pode ser vista na síndrome da rubéola congénita, incluindo distúrbios endócrinos, tais como a doença de Addison, deficiência de hormônio do crescimento e frequência aumentada de diabetes.8

No caso de desencadeante viral de DT1 autoimune, certos vírus (retrovírus em camundongos NOD, vírus da rubéola em hamsters e seres humanos) podem transformar um antígeno de célula beta que existe normalmente em uma forma imunogênica ou podem induzir um novo antígeno, levando a diabetes mellitus insulinodependente com autoimunidade específica para células beta. Além disso, outros vírus podem gerar células T efeitos antígeno-específicos, que podem ter uma reação cruzada com um autoantígeno que é célula beta-específico.9

Uma outra questão levantada nos últimos anos é se a vacinação pode induzir autoimunidade. As reações ao imunógeno ou mecanismos de ativação bystander em indivíduos predispostos. As reações ao imunógeno associadas com imunização incluem a síndrome de Guillain-Barré após a vacinação contra a gripe suína em 1976 e a purpura trombocitopênica imunológica após a vacinação contra sarampo/caxumba/rubéola.10–13

As vacinas podem causar eventos adversos, com efeitos colaterais em sua maioria leves e transitórios. No entanto, reações como hipersensibilidade, indução de infecção e autoimunidade ocorrem. A raridade e a apresentação subaguda dos fenômenos variam de dias a anos.12

No seguinte modelo animal experimental foi examinada a relação causal entre vacinas e achados autoimunes: em filhotes saudáveis de cães imunizados com vacinas comumente administradas, documentou-se uma variedade de autoanticorpos, mas nenhuma doença autoimune foi encontrada. Esses achados também poderiam representar uma ativação policlonal (reação ao adjuvante).

Os mecanismos das reações autoimunes que se seguem a imunizações não foram elucidados. Como mencionado anteriormente, uma das possibilidades é o mimetismo molecular, quando existe uma semelhança estrutural entre um antígeno viral e o autoantígeno.11 A participação de vários fatores ambientais na patogênese de doenças imunomediadas já está bem-estabelecida.

Dessas fatores, aqueles que apresentam uma atividade imune adjuvante, tais como agentes infeciosos, silicone e sais de alumínio, foram associados com doenças imunomediadas definidas e não definidas, tanto em modelos animais quanto em seres humanos. Recentemente, uma síndrome denominada ASIA (do inglês, Autoimmune Syndrome Induced by Adjuvants), consistindo em quatro condições (siliconose, síndrome da guerra do Golfo, síndrome da miofascite macrofágica e eventos pós-vacinais), foi relacionada com exposição prévia a um adjuvante.14 Estudos epidemiológicos são necessários para a obtenção de mais dados sobre as questões aqui levantadas.

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REFERENCES

Prevalence of rubella serum antibody in autoimmune diseases

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ABSTRACT

Introduction: The association between infections and autoimmune diseases (AID) has been well described in the medical literature. Several infectious agents have been implicated as inducers of autoimmune responses, such as Parvovirus B19, Epstein-Barr virus, cytomegalovirus, and hepatitis viruses. Patients and methods: We examined 1,173 sera from patients with 14 different AID and 238 sera from geographically matched healthy controls, for evidence of prior infection with rubella. All samples were tested for the presence of serum antibodies against rubella using the Bio-Rad BioPlex 2200 system. Results: As a group, patients with AID had a higher prevalence of IgM anti-rubella antibodies as compared to healthy controls (11.7% versus 5.4%; \( P = 0.001 \)). The prevalence of IgM anti-rubella antibodies was significantly higher in 5/14 AID, namely in patients with giant cell arteritis (33.3%), primary biliary cirrhosis (24%), antiphospholipid syndrome (20.6%), polymyositis (16%), and inflammatory bowel disease (16%). A similar prevalence of IgM anti-rubella antibodies was detected among controls from different countries. A high prevalence of IgG anti-rubella antibodies was detected among patients with AID (89.9%) and controls. Conclusion: The increased prevalence of IgM anti-rubella antibodies in AID suggests a possible role for rubella in the etiopathogenesis of several AID.

Keywords: rubella, antibodies, autoimmune diseases.

INTRODUCTION

The association of infections with the emergence of autoimmunity has been well described in the medical literature. Viral infections may promote autoimmunity via several different and sometimes combined mechanisms. These mechanisms include: direct cytolysis of virus-infected cells; induction of autoimmune responses to “altered self antigens”; molecular mimicry resulting in the activation of autoreactive T and/or B cells by viral antigens; and bystander activation of autoreactive T cells that may be driven by viral superantigens. In addition, viruses may induce disturbances of the finely tuned balance between T regulatory cells (Tregs) and autoreactive T cells in favor of the autoreactivity, either by specific infection or destruction of Treg or by expansion of autoreactive T cells, and viruses may lead to the activation of innate immunity by triggering toll-like receptors. It could well be that multiple mechanisms need to act in concert to result in disease onset.

Several viruses have been implicated as possible initiators or promoters of autoimmune responses. Viral infections commonly produce transient autoimmune responses, most commonly directed against blood cells, accompanied by transient elevation in autoantibodies titers. Parvovirus B19, Epstein-Barr virus (EBV), cytomegalovirus (CMV), and hepatitis viruses...
are notoriously associated with the induction of autoimmune hemolytic anemia, thrombocytopenia, and arthritis. For the vast majority of infected patients, the clinical symptoms and antibody titers are transient, and decline over several weeks.

Rubella and congenital rubella syndrome (CRS) are caused by rubella virus (RV). Rubella infection usually presents as low-grade fever accompanied by skin rash, conjunctivitis, sore throat, headache, myalgia, anorexia, nausea, tender lymphadenopathy, and soft palate petechiae. Joint involvement is a common manifestation of rubella. Arthritis or arthralgias occurs in up to 50% of adult females, developing soon after the onset of the rash. Transplacental transmission of RV to the fetus during organogenesis leads to severe congenital malformations collectively referred to as CRS. These include sensorineural hearing loss, ocular abnormalities, congenital heart disease, and central nervous system abnormalities.7

Delayed-onset of type-1 diabetes (T1DM) and thyroid abnormalities may develop in the first and second decades of life and represent the most well-known association of rubella with autoimmune diseases (AID). Genetic studies demonstrated that patients with CRS who developed T1DM had a significant increase in HLA-DR3 and decreased HLA-DR2 haplotype, which are characteristic of autoimmunity. In addition, autoantibodies against insulin and islet cells and T-cell abnormalities have been demonstrated in CRS subjects who developed T1DM.

More recent studies have questioned the association of CRS with the development of these autoantibodies. Experimental evidence for a possible causal relationship between rubella infection and T1DM can be derived from the experimental model of T1DM in hamsters, where infection of neonatal hamsters with rubella induces diabetes. As no signs of cytopathological effects can be observed after infection of human islets cells by rubella, one of the most accepted mechanisms for injury is the immune mechanism via molecular mimicry. This is supported by findings of cross-reactivity of T cells from CRS patients affected by T1DM, between rubella virus peptides and GAD protein determinants. Additional features of CRS, such as the late-onset pulmonary and skin disease in infants, lymphocytic infiltration seen in infant pancreas at autopsy, and in some individuals the detection of pancreatic islet cell antibodies and insulin autoantibodies, all support a role for rubella infection on the development of T1DM. Thyroid autoimmunity is also more common in the CRS, providing further support for the concept of rubella-associated autoimmunity.

Due to its many teratogenic effects leading to CRS, rubella infection is a major public health topic. The best strategy to prevent rubella and CRS is vaccination, which has been endorsed by the WHO, and is currently applied all over the world. A single dose of rubella vaccine confers immunity in 95% or more of recipients at 12 months of age or older.

Until now, the exposure to rubella in the past has been evaluated only in small groups of patients with selected AID. In this study we recruited a large cohort of patients with several AID and evaluated their sera for evidence of prior exposure to rubella, and its possible implications.

PATIENTS

Serum samples were collected from 1,173 patients with 14 different AID. These included rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), antiphospholipid syndrome (APS), inflammatory bowel disease (IBD), polymyositis, systemic sclerosis (SSc), primary biliary cirrhosis (PBC), Sjögren’s syndrome (SS), multiple sclerosis (MS), autoimmune thyroid disease (Graves disease and Hashimoto’s thyroiditis), vasculitides including giant cell arteritis (GCA), and pemphigus vulgaris (PV). The control sera were collected from 238 geographically matched healthy controls with similar age and gender distribution. This study was approved by the local Ethical Committees and fulfilled the ethical guidelines of the Declaration of Helsinki (Edinburgh, 2000).

METHODS

All sera were tested for the presence of serum antibodies (IgM and IgG) against rubella using the Bio-Rad BioPlex 2200 system® (Hercules, CA, USA). The BioPlex 2200 is a fully automated, random-access analyzer built on a synthesis of multiplex, magnetic beads, and flow cytometry technologies. At the core of the technology are 25 different populations of 8-μm magnetic beads, which are dyed with two fluorophores for classification purposes. Each bead is coated with specific proteins, according to the different assay being tested, thus representing a different target antigen. Briefly, colored beads coated with different antigens were mixed together, along with the patient’s sample and sample diluent and then allowed to incubate for 20 min at 37 °C.

After a wash cycle, different isotypes of antihuman antibodies, according to the different assay, conjugated to phycoerythrin (PE) were added to the dyed bead and allowed to incubate for 10 min at 37 °C. After removal of excess conjugate, the bead mixture was passed through the detector that identifies the beads based on the fluorescence of the dyes. The amount of antibody bound to the bead was determined by the fluorescence of PE.
Raw data were initially measured as the relative fluorescence intensity and then converted to the fluorescence ratio using a pre-dyed internal standard bead, which is included in every bead set to normalize the detector signal. A series of calibrators were analyzed along with the patient samples to convert fluorescence ratios to antibody concentration units. Two additional control beads were also included in all incubations. A serum verification bead and a blank bead were added to verify the addition of serum to the reaction vessel and the absence of significant nonspecific binding, respectively. Elevated titers of IgG anti-rubella antibodies were determined at 10 IU/mL according to the manufacturer instructions. At the time the tests were performed, the BioPlex kit for IgM was still in developmental stages and was not commercially available. As such, we decided on a cut-off value of two standard deviations above the geographically matched normal control group to define results as positive. The technology applied in this work had already been published and evaluated prior to this study, including in our previous works as well as in other publications.21,22

All data are expressed as means ± confidence interval. The statistical analysis was performed by Mann-Whitney U test. P < 0.05 was considered significant. The Matlab program (Mathworks, Natick, MA, USA) was used for all statistical analyses.

Table 1
Prevalence of IgM and IgG for rubella in autoimmune diseases

<table>
<thead>
<tr>
<th>European AID</th>
<th>No. of patients evaluated (IgM)</th>
<th>IgM levels &gt; 1.8 IU/mL (% (n))</th>
<th>P</th>
<th>No. of patients evaluated (IgG)</th>
<th>IgG levels &gt; 10 IU/mL (% (n))</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>APS</td>
<td>97</td>
<td>20.6% (20)</td>
<td>&lt;0.001</td>
<td>96</td>
<td>97.9% (94)</td>
<td>NS</td>
</tr>
<tr>
<td>SLE-APS</td>
<td>60</td>
<td>11.6% (7)</td>
<td>NS</td>
<td>62</td>
<td>98.4% (61)</td>
<td>NS</td>
</tr>
<tr>
<td>Vasculitides</td>
<td>32</td>
<td>9.4% (3)</td>
<td>NS</td>
<td>31</td>
<td>90.3% (28)</td>
<td>NS</td>
</tr>
<tr>
<td>GCA</td>
<td>21</td>
<td>33.3% (7)</td>
<td>&lt;0.001</td>
<td>29</td>
<td>96.5% (28)</td>
<td>NS</td>
</tr>
<tr>
<td>IBD</td>
<td>119</td>
<td>15.9% (19)</td>
<td>0.015</td>
<td>115</td>
<td>93.9% (108)</td>
<td>NS</td>
</tr>
<tr>
<td>PM</td>
<td>100</td>
<td>16% (16)</td>
<td>0.02</td>
<td>98</td>
<td>95.9% (95)</td>
<td>NS</td>
</tr>
<tr>
<td>Graves</td>
<td>70</td>
<td>5.7% (4)</td>
<td>NS</td>
<td>70</td>
<td>98.6% (69)</td>
<td>NS</td>
</tr>
<tr>
<td>Hashimoto</td>
<td>50</td>
<td>10% (5)</td>
<td>NS</td>
<td>50</td>
<td>90% (45)</td>
<td>NS</td>
</tr>
<tr>
<td>PV</td>
<td>29</td>
<td>13.8% (4)</td>
<td>NS</td>
<td>29</td>
<td>96.5% (28)</td>
<td>NS</td>
</tr>
<tr>
<td>PBC</td>
<td>66</td>
<td>24.2% (16)</td>
<td>&lt;0.001</td>
<td>69</td>
<td>88.4% (61)</td>
<td>NS</td>
</tr>
<tr>
<td>SSc</td>
<td>78</td>
<td>12.8% (10)</td>
<td>NS</td>
<td>80</td>
<td>93.7% (75)</td>
<td>NS</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Columbian AID</th>
<th>No. of patients evaluated (IgM)</th>
<th>IgM levels &gt; 2.6 IU/mL (% (n))</th>
<th>P</th>
<th>No. of patients evaluated (IgG)</th>
<th>IgG levels &gt; 10 IU/mL (% (n))</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLE</td>
<td>119</td>
<td>6.7% (8)</td>
<td>NS</td>
<td>290</td>
<td>90% (261)</td>
<td>NS</td>
</tr>
<tr>
<td>SS</td>
<td>82</td>
<td>4.8% (4)</td>
<td>NS</td>
<td>84</td>
<td>78.6% (66)</td>
<td>NS</td>
</tr>
<tr>
<td>RA</td>
<td>152</td>
<td>8.5% (13)</td>
<td>NS</td>
<td>187</td>
<td>93% (174)</td>
<td>NS</td>
</tr>
<tr>
<td>MS</td>
<td>98</td>
<td>2% (2)</td>
<td>NS</td>
<td></td>
<td></td>
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</tbody>
</table>

RESULTS

The 1,173 patients with AID and their respective controls came from two distinct groups, Western Europeans and Colombian patients, and each group was analyzed separately. Among the 722 Western Europe patients, the AID occurred as follow: primary APS (97), SLE and secondary APS (60), ANCA-positive vasculitides (32), GCA (21), IBD (80 Crohn’s disease and 39 patients had ulcerative colitis), polymyositis (100), Hashimoto’s thyroiditis (50), Graves disease (70), PV (29), PBC (66), and SSc (78). Among the 451 Colombian patients, there were 152 patients with RA, 119 with SLE, 98 with MS, and 82 with SS.

Table 1 summarizes the prevalence of IgM and IgG antibodies to rubella in the different AID and according to geographic origin. Of notice, the prevalence of anti-rubella IgM among all the controls, from the two distinct geographical areas, was low, and did not significantly differ (5.1% and 5.7% P = 0.3). In contrast, as a group, patients with AID had a higher prevalence of IgM antibodies to rubella, 138/1173 patients (11.7%) as compared to 13/238 (5.4%) among all matched healthy controls (P = 0.001).

The prevalence of IgM anti-rubella antibodies was significantly higher in 5/14 different AID, namely in patients with GCA (33.3%), PBC (24%), APS (20.6%), polymyositis...
Rubella infection has been associated with several autoimmune features including transient hematological disturbances, joint diseases, and late-onset T1DM and thyroid abnormalities. As rubella vaccination became the standard of care in the western world, we sought to explore the possible link between rubella past exposure, either by natural infection or by vaccination, with several AID. This is the first study to evaluate the prevalence of rubella past exposure in a very large cohort of patients with a wide spectrum of AID.

Rubella virus (RV) is a single-stranded RNA virus, which has a central core, the nucleocapsid is composed of polypeptide C (C protein), and is surrounded by a lipid-containing envelope. The outer envelope contains several copies of two virus-specific polypeptides (E1, E2), which are important for viral virulence and immunity. Following invasion of the epithelium of the nasopharynx, the RV spreads hematogenously (primary viremia) to regional and distant lymphatics and replicates in the reticuloendothelial system. This is followed by a secondary viremia, when the RV can be recovered from different body sites including lymph nodes, urine, cerebrospinal fluid (CSF), synovial fluid, and lungs. Individuals may acquire the infection from a completely asymptomatic patient or from an individual shedding the virus during the incubation period.

Rubella major manifestations include skin rash, conjunctivitis, sore throat, headache, myalgia, fever, tender lymphadenopathy, and soft palate petechiae. The rash disappears as the humoral immune response develops, parallel also to termination of viremia. Arthritis or arthralgias are common features during or after rubella infection. The arthritis is frequently symmetrical, polyarticular, and most commonly involves metacarpal and proximal interphalangeal joints of the hand, followed by the wrists, knees, and ankles. The arthritis is usually self-limited, however massive effusions may accompany arthritis, and prolonged or relapsing joint symptoms may follow exposure to RV or to rubella vaccine. As RV may directly infect synovial cells, and as arthritis appears concomitantly with the antibody response, immune complexes are probably involved in the pathogenesis of rubella arthritis.

Rare complications of RV infection include encephalitis, post-infectious encephalopathy, Guillain-Barré polyradiculitis, thrombocytopenia purpura and hemolytic anemia. In the CRS, patients typically are afflicted by sensorineural hearing loss, ocular abnormalities, congenital heart disease, and central nervous system abnormalities. Other findings in CRS include hepatosplenomegaly, jaundice, hepatitis, bone lesions, anemia, and thrombocytopenia purpura, and late-onset manifestations consist of thyroid abnormalities and T1DM. These later manifestations support a possible role for RV in the induction of autoimmune disorders.

Most rubella infections lead to long-lasting immunity mediated by antibodies. Humoral and cell-mediated responses are produced against all three structural proteins (C-protein and gp E1 and E2). The capsid protein and E1 and E2 gp contain major virus-neutralizing B-cell and T-helper cell epitopes. As RV has been isolated from patients with prolonged rubella-associated arthritis with apparently adequate antibody responses, several authors have suggested that antibodies alone may sometimes be insufficient to eradicate RV, heightening the important contribution of capsid-specific cytotoxic T cells to the elimination of RV. The method of choice for the diagnosis of rubella is the detection of rubella-specific IgM antibodies in a single serum sample, or a significant (≥ 4-fold) rise in rubella-specific IgG antibody titer between the acute and convalescent serum specimens.

In the present study, a significant increase in anti-rubella IgM antibodies was detected in five AID, namely in APS, GCA, IBD, polymyositis, and PBC patients. These results support the importance of infection exposure in AID.

Several authors have demonstrated an association of rubella exposure, either by natural infection or by vaccination, with autoimmune manifestations such as autoimmune hemolytic anemia, autoimmune thrombocytopenia purpura, arthritis, and arthralgia. Joint involvement has been demonstrated especially in females. Specifically the rubella vaccine has been associated with the development of transient arthralgia (~ 25%), acute arthritis (< 10%), and even rarely with chronic arthritis. Female gender, older age, prior seronegativity, and certain HLA types appear to be risk factors.

Some authors have suggested that prior rubella infection may be associated with RA and with juvenile RA. In a past analysis of antibody profile against rubella in selected groups of patients with juvenile arthritis, rubella vaccine-associated arthritis, SLE and adult RA, antibody titers to rubella in SLE and RA patients were similar to controls, but levels were significantly increased in vaccine-associated arthritis and juvenile arthritis. In addition, in the juvenile group, rubella antigens were detected in the synovial fluid in 33% of patients, leading...
the authors to suggest a possible role for rubella infection at least in a proportion of juvenile arthritis patients. This notion has been reinforced by a recent case reported by Korematsu et al., where a young girl developed abruptly a severe and life-threatening relapse of systemic type juvenile idiopathic arthritis after a prolonged remission, five days after rubella vaccination. The abrupt onset and the temporal relationship to vaccination suggest that molecular mimicry was a plausible mechanism for the severe disease reactivation. Other studies failed to provide conclusive evidence for the role of the rubella in the etiopathology of juvenile RA.

In our large cohort of SLE and RA patients, we could not demonstrate an increased prevalence of rubella past exposure, or increased rubella antibody titers. Our results are in agreement with recent studies which did not demonstrate an increased risk for chronic arthropathy among women vaccinated against rubella. Interestingly, in the subgroup of neuropsychiatric lupus patients, our group has previously reported elevated titers of rubella IgM antibodies, which had a marginal correlation with psychosis and depression.

Our analysis revealed an increased prevalence of anti-rubella IgM in GCA (33.3%). We could not find previous studies on past rubella exposure in GCA patients. Maybe because rubella is usually an infection affecting the young and GCA a disease of the more elderly, this association has not been explored. Our preliminary results should encourage further studies to confirm and explore this possible link.

The association of rubella and liver disease has been documented by several authors. In the past, significant elevated titers of antibodies against rubella have been detected in patients with chronic persistent hepatitis, chronic active hepatitis, and occasionally in acute hepatitis. More recently Kalvenes et al. demonstrated a high antibody response to rubella in active autoimmune chronic hepatitis patients, using three distinct antigen-antibody systems, illustrating an enhanced response to rubella structural proteins in these patients. But with the use of radioimmunoprecipitation assays (RIPA), rubella antibody titers were not increased in PBC patients. These results in PBC patients are in contrast to the other groups of liver diseases, and also with the findings of the present study, and may reflect differences in the population studied and/or in assay technology.

It has been well documented that several infections can be accompanied by increases in circulating antiphospholipid antibodies, including rubella infection. We found a significant prevalence of IgM against rubella in primary APS patients (20.6%), but not among patients with secondary APS (SLE and APS). To our knowledge, this is the first reported association between rubella exposure and primary APS, and as such merits further evaluation. In patients with secondary APS, titers of IgM to rubella were not increased, similar to patients with SLE only.

In the present study we detected elevated titers of IgM to rubella in a significant percentage of patients with polymyositis (16%). We found only one study performed thirty years ago which evaluated antibody titers to viral infections, including rubella, among 20 polymyositis children. In contrast to our results, in this early study performed in children, rubella titers were not elevated. But not only the studies differ in sample size and patients’ age, but studies performed in the 70’s probably included large percentages of non-vaccinated people, and maybe non-exposed people to rubella patients. These differences may explain the discrepancy between results.

In our IBD patients’ group we detected a significant percentage of patients (16%) with increased IgM titers against rubella. Several studies have been conducted addressing the possible relationship between rubella exposure or MMR vaccination and IBD. Most of these studies were epidemiological, and were based on documentation of vaccination or disease, and one study evaluated IgG titers for rubella. All these studies did not find any association between past exposure to measles, mumps or rubella either by natural infection or by vaccination, with the development of ulcerative colitis or Crohn’s disease. Similarly, in our cohort of IBD patients we could not find differences in IgG levels to rubella compared to controls, but IgM titers were significantly elevated. This finding should be confirmed in future studies.

In our large cohort of patients with a wide spectrum of AID, we could not find significant differences in IgG levels against rubella, and these were similar almost across all the diseases and the controls. The high prevalence of IgG seropositivity to rubella reflects the success of the global effort to eradicate CRS by worldwide vaccination. Interestingly, comparing the two control groups, we found that the prevalence was significantly higher in European healthy controls compared to Colombian controls (95.9% versus 89.3%; P = 0.01). This difference can be explained by the earlier introduction of rubella vaccination and the higher coverage of vaccination in Western Europe as compared to Colombia.

Even though rubella vaccination has been available for over 30 years, and global efforts have achieved a high coverage of vaccination in the western world, as reflected by the high percentage of IgG seropositivity among our patients and controls, this study detected significant differences in the titers of IgM to rubella. A significant percentage of selected patients with AID had elevated titers of IgM to rubella. Our results add up to the accumulating evidence on the association of infections and AID, and reinforce the importance of previous exposure to infectious agents on the induction of AID.
Semelhantemente, em nossa coorte de pacientes com DII, não observamos diferenças quanto aos níveis de IgG antirrubéola em comparação aos dos controles, mas os títulos de IgM estavam significativamente elevados. Esse achado precisa ser confirmado em estudos futuros.

Em nossa grande coorte de pacientes com um amplo espectro de DAI não encontramos diferenças significativas nos títulos de IgG antirrubéola, que foram semelhantes em quase todas as outras doenças e controles. A alta prevalência de soropositividade para IgG antirrubéola reflete o sucesso do esforço global para erradicar a SRC por meio de vacinação em todo o mundo. É interessante notar que, comparando-se os dois grupos-controle, descobrimos que a prevalência foi significativamente maior em controles europeus saudáveis em relação a grupos-controle, descobrimos que a prevalência foi significativamente maior em controles europeus saudáveis em relação aos controles colombianos (95,9% versus 89,3%; P = 0,01). Tal diferença pode ser explicada pela introdução mais precoce da vacina rubéolica e maior cobertura de vacinação na Europa ocidental que na Colômbia.

Embora a vacinação contra rubéola esteja disponível há mais de 30 anos e os esforços globais tenham alcançado alta cobertura de vacinação no mundo ocidental, como reflete a alta porcentagem de soropositividade para IgG em nossos pacientes e controles, este estudo detectou diferenças significativas nos títulos de IgM antirrubéola. Uma porcentagem significativa de pacientes selecionados com DAI apresentaram títulos elevados de IgM antirrubéola. Nossos resultados vão ao encontro da evidência acumulada da associação entre infecções e DAI, reforçando a importância da exposição prévia a agentes infecciosos na indução de DAI.

REFERENCES


Stress perception and depressive symptoms: functionality and impact on the quality of life of women with fibromyalgia

Diogo Homann, Joice Mara Facco Stefanello, Suelen Meira Góes, Chris Andreissy Breda, Eduardo dos Santos Paiva, Neiva Leite

ABSTRACT

Introduction: Depression is one of the most frequent psychiatric comorbidities in patients with fibromyalgia (FM), and chronic stress might be one of the triggering events of the characteristic FM symptoms. Objectives: To compare depressive symptoms and stress perception between women with and without FM, in addition to investigate the relationship between those characteristics and the functionality and the impact on the quality of life of those patients. Methods: The study included 20 women with FM (FM group) and 20 healthy women (control group). The following instruments were used: Beck Depression Inventory, Perceived Stress Scale-10, Health Assessment Questionnaire, Fibromyalgia Impact Questionnaire, and Visual Analogue Scale for pain (0–10 cm). Results: The FM group showed higher severity of the depressive symptoms (24.10 ± 11.68) and greater perception of stress (25.10 ± 4.82) as compared with those of the control group (10.20 ± 12.78, P < 0.01; and 15.45 ± 7.29, P < 0.01; respectively). A higher incidence of depressive symptoms was observed in the FM group (75%) than in the control group (25%) (χ² = 10.00, P < 0.01). In the FM group, a positive correlation was observed between the depressive symptoms and perceived stress (r = 0.54, P < 0.05), pain (r = 0.58, P < 0.01), impaired functionality (r = 0.56, P < 0.01), and impact on the quality of life (r = 0.46, P < 0.05). In this group there was also correlation between perceived stress and impaired functionality (r = 0.50; P < 0.05). Pain showed no relationship with perceived stress. Conclusion: The relationship between stress, depression and functionality seems to be part of a complex mechanism, which might affect the quality of life of patients with FM.

Keywords: pain, psychological stress, depression, fibromyalgia.
stands out. It is worth noting that 42% of those individuals identify chronic stress as the trigger event of their symptoms. Acute stressing events are believed to precede the depressive symptoms, because the daily generation of stress might play a role in both maintaining and increasing the probability of the depression recurrence. Thus, stress generation can account for the frequent chronic course of depression.

However, the causal relation between stress and depression in FM does not seem to be linear, but repetitive, and, frequently, patients get stuck in a vicious cycle. The depressive symptoms impair the quality of life of patients with FM by increasing the sensation of pain and the perception of functional disability. In fact, depression is an independent predictor of the physical performance variation in those patients. Thus, factors that intensify the depressive symptoms should be controlled to guarantee the improvement in the quality of life of patients with FM.

Data on the relationship between stress, functionality, depressive symptoms and quality of life of patients diagnosed with FM are still scarce. To identify that relationship is paramount to provide a more effective treatment to that population.

This study aimed at comparing the psychological aspects (perception of stress and depressive symptoms) between women with and without FM and at investigating the possible relationship of those aspects with pain, functionality, and quality of life in women with FM.

METHODOLOGY
Type of the study and subjects of study
This descriptive-comparative cross-sectional study was approved by the Committee of Ethics and Research in Human Beings of the Hospital de Clínicas de la Universidade Federal do Paraná (CEP/HC-UFPR, protocol #2284.178/2010-07), Curitiba, PR, Brazil, and followed the guidelines of the Resolution 196/96 of the National Health Board on research involving human beings.

This study comprised 40 women aged between 29–52 years and divided into the following two groups: 1) FM group – 20 women diagnosed with FM according to the American College of Rheumatology (ACR) criteria and originated from the Rheumatology Outpatient Clinics of the HC/UFPR; 2) control group (CG) – 20 healthy women, paired by age and Body Mass Index (BMI), originating from the community and employees of the UFPR. The selection for the FM group was intentional, based on information from the medical records made available by that Rheumatology Outpatient Clinics. The inclusion criteria established were as follows: age between 20–55 years, BMI between 18.50–39.99 kg/m², neither psychiatric nor neurologic disorders diagnosed, and availability to participate in the study.

After providing written informed consent, the participants underwent anthropometric assessment and physical examination to identify the tender points (TP). Then, the following instruments were applied: Beck Depression Inventory (BDI), Perceived Stress Scale-10 (PSS-10), Health Assessment Questionnaire (HAQ), Fibromyalgia Impact Questionnaire (FIQ), and Visual Analogue Scale (VAS) for pain (0–10 cm).

Anthropometric assessment and physical examination
Body mass (digital scale) and height (fixed wall-mounted stadiometer) were measured according to the Anthropometric Standardization Reference Manual for obtaining the BMI, classified according to the World Health Organization. The TP were assessed in both groups by the same examiner using the digit pressure technique, with strength equivalent to 4 kgf in each painful point, according to the ACR.

Assessment of perceived stress
To assess perceived stress, the PSS-10, proposed by Cohen et al. and validated for the Brazilian population by Reis et al., was used.

The PSS-10 is a self-reported global measure of the degree to which situations in one’s life are appraised as stressful. The scale comprises 10 items relating events and situations that occurred in the last 30 days. Each item is assessed by use of a Likert scale, in which 0 means never, and 4, very often. Of the 10 items, six refer to negative aspects (1, 2, 3, 6, 9, and 10) and four refer to positive aspects (4, 5, 7, and 8). For the final score, the four positive items should be inversely punctuated and, then, all items should be added. The results can vary from 0–40, and a higher score indicates greater perception of stress.

Assessment of the depressive symptoms
The BDI, proposed by Beck et al. and validated for the Brazilian population by Reis et al., was used.

The BDI is a self-reported global measure of the degree to which situations in one’s life are appraised as stressful. The scale comprises 10 items relating events and situations that occurred in the last 30 days. Each item is assessed by use of a Likert scale, in which 0 means never, and 4, very often. Of the 10 items, six refer to negative aspects (1, 2, 3, 6, 9, and 10) and four refer to positive aspects (4, 5, 7, and 8). For the final score, the four positive items should be inversely punctuated and, then, all items should be added. The results can vary from 0–40, and a higher score indicates greater perception of stress.
proved to be a sensitive instrument to assess depression in patients with FM.23

The BDI comprises 21 items that assess depressive attitudes and symptoms, with four options of answer (0–3). The higher the score obtained, the greater the severity of the aspect assessed. The cut-off points depend on both the nature of the sample and the objectives of the study. In non-diagnosed samples, the recommended cut-off points are: ≤ 15 (normal or mild depression), 16–20 (dysphoria), and > 20 (depression).22

Despite different approaches used to identify depression, Gorenstein et al.21 have reported that a score greater than 16 already indicates its possibility.

Assessment of functionality
Functionality was measured by use of the HAQ proposed by Fries et al.24 in its version translated and validated for the Brazilian population.25 The HAQ is divided into the following eight components: dressing and grooming; arising; eating; walking; hygiene; reach; grip; and common daily activities. Each component is approached in two or three questions, in a total of 20 questions. Each question offers four answering options (0–3), and the individual should pick one. The higher the score, the greater the individual’s disability. A final score was categorized as follows: 0–1, mild to moderate difficulty; 1–2, moderate difficulty to severe disability; and 2–3, severe to very severe disability.26

Assessment of the impact on quality of life
To assess the impact of FM on the participants’ quality of life, the FIQ proposed by Burckhardt et al.27 and translated and validated for the Brazilian population was used.28 The FIQ is a specific questionnaire developed to assess the FM impact on the quality of life of the patients and is composed of the following ten items: physical functioning; well-being; work missed; work disability; pain; fatigue; stiffness; sleep; anxiety; and depression.

The FIQ questions should be answered based on the respondent’s perception of the last seven days. The final score varies from 0–100, and the highest score indicates the greatest impact of FM on quality of life.

Assessment of pain severity
A 10-cm VAS, in which 0 stands for lack of pain and 10 stands for unbearable pain, was used. In the present study, the severity of pain (milder, stronger and intermediate) was assessed in the last week and at the time of the assessment. The mean of four measurements reflects pain severity more accurately than one single measure, avoiding both underestimating and overestimating that characteristic by the individuals.29

Statistical treatment
The data were analyzed by using the Statistica software (Statsoft Inc., version 7.0). The normality of the data was checked by using the Shapiro-Wilk test, and the homogeneity of the variances, when comparing both groups, by using the Levene’s test. Pearson correlation and the independent t test were used for the parametric data, and the Spearman correlation and the Mann-Whitney U test, for the non-parametric data. The chi-square test was used to assess differences regarding proportions. The significance level of P ≤ 0.05 was adopted.

RESULTS

Table 1 shows the general characteristics of the sample. The FM group had a greater number of TP, greater pain severity, more difficulty in performing daily activities, and a greater impact on the quality of life as compared with those of the CG.

The BDI scoring is shown in Figure 1. Patients with FM have more severe depressive symptoms, and the proportion of those patients with possible depression (≥ 16 points) was 75% versus 25% in the CG (χ² = 10.00; P < 0.01).

The PSS-10 scoring is shown in Figure 2. The FM group had a higher perception of stress (FM group: 25.10 ± 4.82 versus CG: 15.45 ± 7.29).

Table 2 shows the correlations between the variables assessed in the study for the FM group. Although stress had a positive correlation only with greater difficulty in performing daily activities and greater depression, depression was found to correlate with most of the variables studied (pain, functionality, stress, and impact on quality of life).
DISCUSSION

The proportion of individuals with FM with depressive symptoms was significantly higher as compared with those of the CG (75% and 25%, respectively). In addition, the impact on quality of life was higher in the FM group (68.88 ± 15.04 versus 22.66 ± 14.05 on the CG). Recently, Aguglia et al. have found similar results, reporting that 83.3% of the patients had depressive symptoms and worse quality of life. Studies carried out in Brazil have also found a high prevalence of depressive symptoms. Martinez et al. have found that 80% of the patients with FM reported more depressive symptoms as compared with healthy individuals (12%). Berber et al. have concluded that approximately two thirds of the patients studied had a depressive condition.

The results found in the present study differed from those by Santos et al., who have used the same instrument to assess depressive symptoms in patients with FM. While in this study the mean results (24.10 ± 11.68 for the FM group, and 10.20 ± 12.78 for the CG) indicate a depressive setting, those authors have indicated a dysphoric setting (17.75 ± 11.23 for patients, and 9.50 ± 6.44 for controls).

The depressive symptoms correlated with a higher number of the variables studied. That is, the higher the intensity of the depressive symptoms, the higher the pain severity, the difficulties in performing the daily chores, the perceived stress, and the negative impact on the quality of life. The relationship between the depressive symptoms and the impairment in the quality of life, mainly related to aspects of physical functionality and pain perception, has also been reported in a previous study carried out in Brazil.

Some authors have suggested that, due to the close relationship between certain symptoms of FM, mainly pain and psychiatric disorders, a pathophysiological overlapping for those processes might exist. Evidences indicate that certain brain areas involved in the generation of emotions are also involved in pain modulation. Thus, depression could amplify painful signs. In addition, depression is associated with changes in some neurotransmitters, which can reduce the modulatory effect of the pain inhibitory system.

Individuals suffering from chronic pain and depression, including those with FM, have reduced functionality as compared with those without depression.

In the present study, the impairment in performing daily chores differed between both groups, with the FM group showing moderate difficulty to severe disability. In addition, among the patients, the higher the pain severity reported, the greater the difficulty in performing daily activities. In fact,

Table 2

<table>
<thead>
<tr>
<th>No. of TP</th>
<th>Pain</th>
<th>HAQ</th>
<th>FIQ</th>
<th>PSS-10</th>
<th>BDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of TP</td>
<td>–</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pain</td>
<td>0.11</td>
<td>–</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HAQ</td>
<td>0.33</td>
<td>0.47*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FIQ</td>
<td>0.36</td>
<td>0.48*</td>
<td>0.41</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PSS-10</td>
<td>0.07</td>
<td>0.32</td>
<td>0.50*</td>
<td>0.32</td>
<td>–</td>
</tr>
<tr>
<td>BDI</td>
<td>0.31</td>
<td>0.58**</td>
<td>0.56*</td>
<td>0.46*</td>
<td>0.54*</td>
</tr>
</tbody>
</table>

TP: tender points; HAQ: Health Assessment Questionnaire; FIQ: Fibromyalgia Impact Questionnaire; PSS-10: Perceived Stress Scale; BDI: Beck Depression Inventory. *P ≤ 0.05; **P ≤ 0.01.

Figure 1
Comparison of the severity of the depressive symptoms between the group of patients with fibromyalgia (FM) and the control group (CG).

Figure 2
Comparison of the stress perceived between the group of patients with fibromyalgia (FM) and the control group (CG).
pain accounts for generating a 40% variation in functionality, when assessed by using the HAQ.36

Not only pain intensity seems to influence that relationship, but also the number of TP, because a significant correlation was found between that number and the difficulty in performing daily functional activities (according to the HAQ).37 However, in the present study, no relationship was found between the TP and any other variable assessed, contrary to other findings, in which the presence of the TP can reflect a measure of altered response to stress,38,39 leaving areas of the body more sensitive.

The patients assessed had a higher perception of stress, confirming the findings of other authors, who have reported an important impact of that variable on FM and the severity of its symptoms.40 Patients with FM have shown a greater perception of stress as compared with healthy controls,41 and a greater perception of psychological stress as compared with patients with other chronic pain types.35

When investigating the relationship between pain and stress in patients with FM and healthy individuals, Ferreira et al.42 have found no significant difference in the number of stressing events between both groups, attributing their result to the way patients cope with stress, and not only to the intensity of the events experienced. Differently, Becker et al.43 have reported a relationship between high levels of stress and that syndrome, in addition to the existence of an interaction between the apo-lipoprotein E gene polymorphism and stress in FM.

In the present study, the stress perceived in the FM group showed no direct relationship to pain. This suggests that the degree patients perceive the life situations as stressing does not directly relate to the fact that they feel pain. However, stressful experiences have been associated with changes in pain threshold,44 depending on the type of stress experienced (physical or emotional), as well as on its intensity and duration.45

The relationships between reduced functionality, stress, and depression found in the present study can indicate that the impairment in performing daily activities was a stress-generating factor for the FM group. Considering that the effects of negative chronic conditions seem to amplify the association between acute daily events and depression,49 the relationship between functionality impairment and higher perception of stress can make patients with FM more susceptible to the appearance of depressive symptoms. Knowing the relationship between those variables and their intensity is important to elaborate adequate strategies for the treatment of FM.

CONCLUSIONS

Patients with FM have reduced functionality, greater perception of stress, and more severe depressive symptoms than healthy individuals.

Greater pain severity, reduced functionality, greater perception of stress, and greater impact on the quality of life showed a direct relationship with depressive symptoms in patients with FM. The impaired functionality in that group related to a greater perception of stress, but not to the quality of life. Stress showed no relationship to pain severity in patients with FM.

It is worth noting the relationship between stress, depression and functionality as part of a complex mechanism that can interfere with the quality of life of patients with FM.

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REFERÊNCIAS


Frailty syndrome in the community-dwelling elderly with osteoarthritis

Rita de Cássia Corrêa Miguel1, Rosângela Corrêa Dias2, João Marcos Domingues Dias2, Silvia Lanziotti Azevedo da Silva1, Paulo Roberto Menicucci Filho4, Tatiana Moreira S. Ribeiro5

ABSTRACT

Objective: To characterize and compare community-dwelling elderly with knee and/or hip osteoarthritis (OA), focusing on the frailty syndrome. Method: Cross-sectional study of the elderly with knee and/or hip OA, using a subsample from the study of frailty in the Brazilian elderly (FIBRA), assessing the following: sociodemographic characteristics, comorbidity, medications, depression, anthropomorphic data, falls, pain, stiffness, physical function, and frailty. The subjective assessment of health was also performed. Results: The final sample comprised 58 elderly (mean age, 74 ± 5.5 years) as follows: 17 (29.31%) non-frail, 28 (48.28%) pre-frail, and 13 (22.41%) frail. The frail elderly received more medications than the non-frail ones (7.00 ± 2.00 and 4.00 ± 2.00, respectively; P = 0.001). The mean Body Mass Index was lower in the non-frail elderly as compared with those of the pre-frail and frail ones (27.00 ± 4.50 kg/m², 30.00 ± 4.00 kg/m², and 34.00 ± 8.00 kg/m², respectively; P = 0.018). Depression was more prevalent in the frail group. Compared to the previous year, there was a difference in the health status of the groups as follows: 64.3% of the pre-frail elderly and 52.9% of the non-frail elderly considered that their health status remained unchanged (P = 0.016). When comparing the current physical activity levels with those of the previous year, the pre-frail and frail elderly reported a worsening (P = 0.010). Regarding physical function and fall-related self-efficacy, the frail elderly were worse than the others (P = 0.023 and 0.017, respectively). There were no significant differences between the groups for the remaining items analyzed. Conclusion: The elderly with OA and frailty use more medications, are more obese and depressed, have a poorer perception of their own health and of their level of activity as compared with that of the previous year, have a worse fall-related self-efficacy, and worse physical function.

Keywords: frail elderly, knee osteoarthritis, hip osteoarthritis, disable people.

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INTRODUCTION

The increase in the population’s life expectancy has a significant impact on health conditions, morbidity and functional limitations among the elderly.1 Osteoarthritis (OA) is strongly associated with age and is the most common rheumatic disease, affecting approximately 16% of the Brazilian population.2–4 Of the large joints, knees and hips are the most often affected by OA.2 Its clinical manifestations vary and include pain, stiffness, reduction in muscle strength, joint instability, deformities, and gait changes.2,3 Such manifestations can lead to functional limitation, disability and falls.2,4,5
Frailty is a distinct clinical syndrome in which there is a reduction in the body’s physiological reserves and homeostatic capacity to resist to stressors, due to the cumulative decline of the physiological systems, comprising mainly sarcopenia, neuroendocrine dysregulation, and immune dysfunction.6–9 Fried et al.6 have proposed the operational definition of a frailty phenotype, which was used in the present study. Frail elderly are more susceptible to adverse outcomes, such as functional limitation, falls, institutionalization, hospitalization, and increased mortality.6

OA is considerably prevalent in studies about frailty, with which it has a significant association.10,11 Nevertheless, in addition to the isolated impact of frailty and OA on the functional capacity and quality of life of the elderly,4,6 no study characterizing the elderly with OA based on the frailty phenotype has been found in the databases assessed. Thus, this study aimed at characterizing community-dwelling elderly with knee and/or hip OA regarding sociodemographic, clinical, and functional aspects, with emphasis on the frailty syndrome.

**MATERIALS AND METHODS**

This cross-sectional observational study was approved by the Committee on Ethics and Research (0062.0.203.000-10). All participants provided written informed consent.

From the database of the study *Perfis de Fragilidade em Idosos Brasileiros Residentes na Comunidade* (Rede FIBRA, central area of the city of Belo Horizonte) with 607 community-dwelling elderly, all individuals who answered affirmatively to the question “Have you been diagnosed with arthritis or rheumatism by a physician?” (162 elderly) were selected for contact. The inclusion criteria were age over 65 years and meeting the American College of Rheumatology criteria for knee and/or hip OA.2,3,12 The exclusion criteria were: cognitive deficit identified in the Mini-mental State Examination,13 previous arthroplasty, diagnosis of systemic rheumatic diseases, impossibility to walk independently, and lack of handgrip strength.

The elderly diagnosed with, or suspected of having, knee and/or hip OA were invited to participate in the study and visited in their homes. During the visits, the clinical diagnosis of knee and/or hip OA was assessed and those who did not meet the criteria proposed in the study were excluded. Once the diagnosis of OA was confirmed, the WOMAC (Western Ontario and McMaster Universities) questionnaire,14 specific to assess pain, stiffness, and physical function domains in patients with knee and/or hip OA, was applied. Then, the Rede FIBRA questionnaire, containing items that assess sociodemographic characteristics, comorbidities, perceived physical health, Body Mass Index (BMI), falls (self-reported and Falls Efficacy Scale – FES-I-Brasil,15 the later according to cut-off points defined by Camargos et al.15 and total score), and depression (self-reported and Geriatric Depression Scale – GDS),16 was used. In addition, that questionnaire contains questions and measures that allow the characterization of the elderly according to the frailty phenotype proposed by Fried et al.,6 as follows: unintentional weight loss (≥ 4.5 kg), self-reported exhaustion (assessed by use of the questions: “Have you felt you had to make an effort to deal with your usual chores?” and “Have you dropped many of your activities and interests?”), the answers “frequently” or “always” to any of those questions scored one point), weak grip strength (assessed by use of the JAMAR dynamometer, and with the adoption of the cut-off points proposed by Fried6 and adjusted for gender and BMI), low physical activity (assessed by use of the Minnesota Leisure Time Activities Questionnaire, according to which, women with a week energy expenditure < 270 kcal and men with a week energy expenditure < 383 kcal were considered fragile for that item), and slow walking speed (time spent, in seconds, to cover a 4.6-m distance at a usual auto-selected speed; the cut-off points proposed by Fried6 and adjusted for gender and height were adopted). According to the number of those characteristics, the elderly were classified as frail, with at least three of those characteristics; pre-frail, with one or two; and non-frail, with none.

The elderly included in the study were invited to undergo radiography of the joints affected at a specialized clinic. The analyses were performed by the same examiner, who was unaware of the elderly’s clinical data. Radiographic OA was classified according to the Kellgren-Lawrence grading system.17

**Statistical analysis**

The descriptive analysis of the data collected was performed. The chi-square test was used to assess the differences between the frailty levels for the categorical variables.

When the expected frequency was lower than five, Fisher exact test was used. The continuous variables were assessed by use of analysis of variance (ANOVA) with Tukey multiple comparisons, when data had a normal distribution. When the normality assumption was violated, the Kruskal-Wallis test was used with Mann-Whitney post-tests. All analyses were
performed with the SPSS software, version 16.0, with 95% confidence ($\alpha = 5\%$).

RESULTS

Of the 162 elderly selected, 12 (7.4%) could not be contacted. Of the remaining 150, 37 (24.6%) had other rheumatic diseases, 14 (9.3%) denied the presence of rheumatism, and 99 (66.6%) had symptoms of knee and/or hip OA. Of those 99, nine (9.09%) refused to participate, and the others were assessed. Nineteen elderly (19.2%) were excluded, 13 of whom (13.2%) due to arthroplasty, two (2.0%) due to dementia, three (3.0%) who could not walk independently, and one (1%) who could not undergo the handgrip test. Thus, 71 elderly, who met the clinical criteria for knee and/or hip OA, were included and assessed. Later, 13 of them (18.3%) were excluded from the sample as follows: 10 (14.08%) who refused to undergo radiography and three (4.2%) who had no radiographic changes. Thus, the final analysis comprised 58 elderly: four (6.9%) men and 54 (93.1%) women.

<table>
<thead>
<tr>
<th>Table 1</th>
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<tbody>
<tr>
<td>Descriptive analysis of the total sample (n = 58) and of the frailty groups, and difference between frailty groups</td>
</tr>
<tr>
<td>Variable</td>
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<td>-------------------------------</td>
</tr>
<tr>
<td>Gender</td>
</tr>
<tr>
<td>Male</td>
</tr>
<tr>
<td>Female</td>
</tr>
<tr>
<td>Marital status</td>
</tr>
<tr>
<td>Married</td>
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<tr>
<td>Single</td>
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<tr>
<td>Divorced/separated</td>
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<tr>
<td>Widower</td>
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<tr>
<td>Color/ethnicity</td>
</tr>
<tr>
<td>White</td>
</tr>
<tr>
<td>Black</td>
</tr>
<tr>
<td>Mixed heritage or mulatto</td>
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<tr>
<td>High school</td>
</tr>
<tr>
<td>University</td>
</tr>
<tr>
<td>Live alone</td>
</tr>
<tr>
<td>Yes</td>
</tr>
<tr>
<td>No</td>
</tr>
<tr>
<td>Smoking habit</td>
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<td>Yes</td>
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<tr>
<td>No</td>
</tr>
<tr>
<td>Alcohol drinking</td>
</tr>
<tr>
<td>Never</td>
</tr>
<tr>
<td>Once/month or less</td>
</tr>
<tr>
<td>2-4 times/month</td>
</tr>
<tr>
<td>2-3 times/week</td>
</tr>
</tbody>
</table>

NF: non-frail; PF: pre-frail; F: frail.

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Table 2
Association tests and P values for the variables of subjective health assessment according to the classification of frailty

<table>
<thead>
<tr>
<th>Variable</th>
<th>NF</th>
<th>%</th>
<th>PF</th>
<th>%</th>
<th>F</th>
<th>%</th>
<th>P</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
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<td>n</td>
<td></td>
<td>n</td>
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<tr>
<td>Health self-assessment</td>
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<td></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Excellent</td>
<td>0</td>
<td>0.0</td>
<td>4</td>
<td>14.3</td>
<td>0</td>
<td>0.0</td>
<td>0.200</td>
<td>Chi-square</td>
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<tr>
<td>Good</td>
<td>9</td>
<td>52.9</td>
<td>10</td>
<td>35.7</td>
<td>4</td>
<td>30.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fair</td>
<td>6</td>
<td>35.3</td>
<td>13</td>
<td>46.4</td>
<td>8</td>
<td>61.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poor</td>
<td>2</td>
<td>11.8</td>
<td>0</td>
<td>0.0</td>
<td>1</td>
<td>7.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Very poor</td>
<td>0</td>
<td>0.0</td>
<td>1</td>
<td>3.6</td>
<td>0</td>
<td>0.0</td>
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<tr>
<td>Comparison of health with others of the same age</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Equal</td>
<td>3</td>
<td>17.6</td>
<td>9</td>
<td>32.1</td>
<td>1</td>
<td>7.7</td>
<td>0.301</td>
<td>Fisher exact</td>
</tr>
<tr>
<td>Better</td>
<td>13</td>
<td>76.5</td>
<td>15</td>
<td>53.6</td>
<td>9</td>
<td>69.2</td>
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<tr>
<td>Worse</td>
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<td>5.9</td>
<td>4</td>
<td>14.3</td>
<td>3</td>
<td>21.1</td>
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<td>Current health x previous year</td>
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<td></td>
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<td>Equal</td>
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<td>6</td>
<td>21.4</td>
<td>1</td>
<td>7.7</td>
<td>0.016*</td>
<td>Fisher exact</td>
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<td>11.8</td>
<td>4</td>
<td>14.3</td>
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<td>46.2</td>
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<tr>
<td>Worse</td>
<td>6</td>
<td>35.3</td>
<td>18</td>
<td>64.3</td>
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<td>Health care</td>
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<td>17.9</td>
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<td>38.5</td>
<td>0.301</td>
<td>Fisher exact</td>
</tr>
<tr>
<td>Good</td>
<td>11</td>
<td>64.7</td>
<td>17</td>
<td>60.7</td>
<td>5</td>
<td>38.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fair</td>
<td>5</td>
<td>29.4</td>
<td>5</td>
<td>17.9</td>
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<td>23.1</td>
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<tr>
<td>Poor</td>
<td>0</td>
<td>0.0</td>
<td>1</td>
<td>3.6</td>
<td>0</td>
<td>0.0</td>
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<tr>
<td>Activity level as compared with that of the previous year</td>
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<td></td>
<td></td>
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<tr>
<td>Equal</td>
<td>12</td>
<td>70.6</td>
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<td>32.1</td>
<td>2</td>
<td>15.4</td>
<td>0.010*</td>
<td>Fisher exact</td>
</tr>
<tr>
<td>Better</td>
<td>0</td>
<td>0.0</td>
<td>5</td>
<td>17.9</td>
<td>1</td>
<td>7.7</td>
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<td></td>
</tr>
<tr>
<td>Worse</td>
<td>5</td>
<td>29.4</td>
<td>14</td>
<td>50.0</td>
<td>10</td>
<td>76.9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NF: non-frail; PF: pre-frail; F: frail. *Statistically significant (95% confidence).

The subjective assessment of health (Table 2) showed an association of frailty with the following: the comparison of current health with that of the previous year; and the current activity level as compared with that of the previous year. The other items showed no significant differences between the groups.

The elderly had, on average, 3 ± 1 distinct comorbidities, and no significant difference was found between the frailty classes (P = 0.326). In isolation, only depression showed a significant association with frailty. Only three (17.6%) NF elderly reported depression as compared with 15 (53.6%) PF elderly and seven (61.5%) F elderly (P = 0.041).

Using the GDS, nine (52.9%) NF elderly and 20 (71.4%) PF elderly showed no symptoms of depression. Regarding the F elderly, eight (61.5%) had symptoms. However, Fisher exact test showed no significant association between frailty and the presence of depression according to the GDS (P = 0.118).

Regarding the number of medications used (Table 3), a significant difference between the frailty classes was observed (P = 0.001). The NF elderly differed from the PF and F elderly (P = 0.038 and P = 0.001, respectively). No difference was observed between the PF and F elderly (P = 0.102).

Regarding the BMI (Table 3), the mean for the 58 elderly was 30.00 ± 6.00 kg/m². When assessed in each group, the mean BMI differed significantly between the frailty classes (P = 0.018). The mean BMI of the NF elderly was 27.00 ± 4.5 kg/m², and that of the F elderly was 34.00 ± 8 kg/m². The multiple comparisons evidenced that PF and F elderly were similar regarding the BMI (P = 0.138). On the other hand, the NF elderly differed from both the PF (P = 0.041) and F (P = 0.013) elderly.

Assessing the relationship between the number of falls reported in the last year and the frailty level, in the F group seven elderly (53.8%) reported two or more falls, and five (38.5%) reported none. On the contrary, in the NF group, only two elderly (11.8%) reported two or more falls, as compared with 13 (76.5%) who reported no fall. However, no significant difference between falls and frailty was observed. When comparing the FES-I classification and frailty, the higher percentages of recurring falls were found among the PF (88.2%) and F (76.9%) elderly as compared with that among the NF (41.2%) elderly, but no significant difference was found between non-fallers, fallers and recurring fallers.
On the contrary, when assessing the FES-I scores (Table 3), the multiple comparisons indicate that the PF and NF elderly were similar (P = 0.851), while the F elderly differed from both the PF (P = 0.014) and NF (P = 0.005) elderly.

In addition, the WOMAC index was assessed in its three domains (Table 4). A significant difference was evidenced in the physical function domain (P = 0.023). The multiple comparisons indicated a similarity between the NF and PF elderly (P = 0.159), as well as between the PF and F elderly (P = 0.365). A significant difference was observed between the two extremes: the F and NF elderly (P = 0.019).

Of all the elderly studied, 54 (93%) had knee OA. The median duration of symptoms was 54 months (P25 = 36; P75 = 165) for the NF elderly, 60 months (P25 = 48; P75 = 150) for the PF elderly, and 120 months (P25 = 60; P75 = 210) for the F elderly. Despite the highest median for the F elderly, no significant difference was observed between the frailty levels (P = 0.37).

The distribution of the NF elderly with knee OA according to the Kellgren-Lawrence grading scale was as follows: grade I, six (37.5%); grade II, four (25%); and grade III, six (37.5%). That distribution of the PF elderly was as follows: grade I, five (20%); grade II, seven (28%); and grade III, 13 (52%). Regarding the F elderly, that distribution was as follows: grade I, two (15.4%); grade II, six (46.2%); grade III, three (23.1%); and grade IV, two (15.4%). The Fisher exact test showed no significant association between frailty and the Kellgren-Lawrence grading scale.

Only seven elderly in the sample (12%) were diagnosed with hip OA, with a median duration of symptoms of 90 months (P25 = 12; P75 = 60) for the NF, of 96 months (P25 = 24) for the PF, and of 96 months for the F elderly. No significant difference was observed between that variable and the frailty levels (P = 0.775). In addition, the Kellgren-Lawrence grading scale showed no significant differences between the frailty levels (P = 1.00).

**DISCUSSION**

This study aimed at assessing the characteristics of the elderly with knee and/or hip OA, focusing on the frailty syndrome.

A significantly higher number of women comprised our sample, and the first explanation for that might be the source of the sample data, in which women were the majority (404 women and 203 men). In addition, it has been reported that knee OA,3,18 which also comprised most of the study sample, is more prevalent in women. Another aspect relates to the question that led us to those elderly, regarding their diagnosis with arthritis or rheumatism by a physician. Such question may represent a bias, because studies have shown that women seek medical care more often than men do,19,20 and, thus, it is believed that women are more likely to have diseases diagnosed by a physician as compared with men.

The percentage of the F elderly found (22.41%) differs from those in the literature. Fried et al.6 have reported 6.9% of F elderly. Cesari et al.10 have reported 8.8%, and Santos21 has found 13.2% of those individuals. Even when compared with the findings by Santos-Eggimann et al.,22 who have assessed frailty in 10 European countries and have found a mean of 17% of F elderly, the prevalence found in the present study remains greater. It is believed that the fact that this study’s sample comprised only elderly with OA might...
have influenced the results, since a significant association between OA and frailty has already been reported.\textsuperscript{10,31} The higher number of women in the present study might also be an explanation, since there is evidence that they tend to be more frail than men.\textsuperscript{6,23} Nevertheless, the prevalence of frail female elderly in the Cardiovascular Health Study (CHS) was 7.3%\textsuperscript{6} and in The Women’s Health and Aging Studies (WHAS), 11.3%.\textsuperscript{21} Another explanation would be Brazilian’s ethnic mixture since its origin. In the study by Cesari et al.,\textsuperscript{10} while Switzerland and Sweden had 5.8\% and 8.6\% of F elderly, respectively, Italy and Spain had 23\% and 27.3\%, respectively.\textsuperscript{22} In an attempt to explain those differences, a study with Afro-Americans (AAM) and European-Americans (EA) has assessed the percentage of elderly in each frailty group using two different cut-off points: one unadjusted for ethnicity and the other adjusted for ethnicity.\textsuperscript{24} When the unadjusted cut-off point was used, a greater percentage of frail individuals was found in the AAM group. That difference disappeared when the cut-off point adjusted for each ethnicity was used. At least part of that difference is believed to occur due to differences in the BMI and height among ethnicities, variables used to establish cut-off points for grip strength and walking speed, respectively.\textsuperscript{24} Along that same line, Santos,\textsuperscript{21} assessing Brazilian older adults, has also reported a difference among the frailty groups after adjusting the Fried’s cut-off points for the population studied. While for the original cut-off points the prevalences of frailty and PF were 13.27\% and 49.29\%, respectively, after adjusting, both groups showed a reduction, passing to 10.6\% and 43.36\% for F and PF groups, respectively.\textsuperscript{22} However, even if the difference in the cut-off point explains part of the present study’s findings, the frailty prevalence, as compared with the unadjusted data of Santos,\textsuperscript{21} remains higher.

Regarding the subjective health assessment, the items analyzing the current health status and activity level as compared with those of the previous year showed an association with frailty. The number of the F elderly reporting worsening of their health status as compared with that of the previous year was the same of those reporting an improvement. On the contrary, in the PF group, most elderly reported worsening of their health status as compared with that of the previous year. Regarding the current physical activity level as compared with that of the previous year, both the F and PF elderly reported worsening. The cross-sectional characteristic of this study does not allow stating the causes of those changes, but, based on the literature, one can speculate about some of them.

Health self-assessment is known to predict, in a robust and consistent way, mortality and functional decline,\textsuperscript{25} and frailty has already been observed to relate to those aspects. Thus, the first justification could be the dynamic characteristic of frailty,\textsuperscript{26} as follows: the elderly who currently report worsening could be, in the previous year, in another frailty category, considering the frailty phenotype.\textsuperscript{6} That, however, does not explain the percentage of the F elderly reporting an improvement in their health status. The functional capacity has a floating characteristic,\textsuperscript{27,28} which might explain the reported improvement by part of the F elderly, who, despite remaining in the same frailty category, might have moved to a better level of functional capacity, and, thus, acquired a better perception of health. It might still explain the worsening previously described, since the transition can occur in both directions. In addition to the questions related to frailty, pain in OA has also floating characteristics,\textsuperscript{29} which can justify part of the changes in self-perception.

Contrary to that reported in the literature,\textsuperscript{6,11,30} this study showed no significant difference in the number of comorbidities among the frailty groups (the mean number was the same in all three groups). Although this does not corroborate the findings of other studies,\textsuperscript{6,11,30} the lack of association between comorbidity and frailty emphasizes the concept that frailty is a distinct clinical syndrome.\textsuperscript{6,7} On the other hand, the fact that OA is a disease with a high comorbidity rate\textsuperscript{31,32} might have hindered the identification of differences among the groups.

Reporting depression diagnosed by a physician was associated with frailty. However, when the presence of depressive symptoms was assessed by the GDS instrument,\textsuperscript{16} no significant difference was observed, although the F group showed a higher prevalence. It is believed that part of the elderly who answered “yes” to the question “Have you been diagnosed with depression by a physician?” might have no more symptoms of depression because of the treatment, and, thus, might not have been screened as positive in the GDS.\textsuperscript{16} Other studies\textsuperscript{6,11,30} have reported an association between frailty and symptoms of depression. Two of those studies have excluded from their samples the elderly on antidepressants.\textsuperscript{6,30} Thus, the elderly of those studies who could have depression were those not being treated, which might have allowed the instruments used (Center for Epidemiological Studies-Depression – CES-D and GDS) to detect a greater number of cases, and the association between depression and frailty could be perceived.\textsuperscript{6,30} Thus, we believe that the non-exclusion of the elderly with depression from
the present study made the criterion “depression diagnosed by a physician” more sensitive to demonstrate the association between depression and frailty as compared with the GDS.

Another important aspect well-described in the literature is that OA, depression and frailty, in isolation, are risk factors for incapacity.\(^{4,6,7,33,34}\) In addition, depression has been related to worse functioning in the elderly with OA.\(^{35,36}\) The present study, in which all the elderly have OA, showed that the F elderly are even more depressed than the NF elderly. Thus, the elderly diagnosed with simultaneous OA, depression and frailty should be carefully considered, because they are at higher risk of developing future incapacity.

Although this study found no association between comorbidity and frailty, it showed that the F elderly use a significantly greater number of medications as compared with the NF elderly. One explanation for that finding could be that the severity rather than the number of comorbidities might be more associated with frailty. The higher number of medications might be one way of reflecting the severity of comorbidities. Another aspect reported in the literature is that some adverse effects of the medications can contribute to frailty.\(^{37}\) However, the present study does not allow confirming those hypotheses, and further studies are required to better clarify that question.

The elderly of the present study tended towards obesity, which is a known risk factor for the development and progression of OA\(^{38,39}\) and has effects on the progression of the functional limitation and incapacity of the elderly with that condition.\(^{4,40}\) Some studies have evidenced an association between obesity and frailty,\(^{41,42}\) as well as a greater risk of incapacity in the elderly with sarcopenic obesity.\(^{41,42}\) When assessed according to frailty group, the BMI proved to be significantly greater in the PF and F groups than in the NF group. Those findings suggest that the elderly with OA and frailty have a greater tendency towards obesity, and, in the presence of the three conditions, a higher risk for functional limitation.

The assessment of the number of falls in the last 12 months showed no significant difference among the groups. However, although non-significant, the F group showed a tendency towards a greater percentage of elderly with at least two falls, and recurring falls are the ones that most predict negative outcomes.\(^{41}\) The results of the present study differ from those of other studies,\(^{6,30}\) which show an association between fall and frailty. Because knee and/or hip OA is a predisposing factor to falls, that may have been one of the reasons for not finding an association with frailty in this study, which strengthens the multifactorial character of the etiology of falls.\(^{41}\) Another question is that the elderly can both forget their falls and neglect their occurrence,\(^{44}\) which influences the results obtained in the investigation.

The outcome “fall” was also assessed by use of the FES-I-Brasil.\(^{17}\) When classified as non-fallers, fallers and recurring fallers, no significant difference was observed among the groups, although recurrent falls were more common in the F and PF groups. When the total scores were used, an association was found between the F group and higher scores, evidencing that the elderly with OA and frailty are more concerned about falling, indicating a lower fall-related self-efficacy. An explanation for those apparently discordant results would be that the original cut-off points for the classification of non-fallers, fallers and recurring fallers in the sample of the adaptation study of FES-I to Brazil do not have the same sensitivity in a sample in which all the elderly have knee and/or hip OA — thus, the total score might be more appropriate for the present study. A tendency towards a greater percentage of elderly classified as recurring fallers in the F and PF groups is observed, as is an association between F elderly and worse fall-related self-efficacy when using the total score of the FES-I. Thus, it is worth noting that both worse fall-related self-efficacy and recurring falls might lead to physical activity restriction and worse quality of life.\(^{15,46}\)

Pain, stiffness and physical function were assessed by use of the WOMAC questionnaire,\(^{14}\) and only physical function showed an association with frailty, having the F group the greatest, and thus worse, mean. This shows that the F elderly with OA of this sample have worse function and might be at a higher risk for progressing with incapacities and dependence than the NF elderly with OA.

Although no significant difference was observed for the “pain” domain, the F elderly had a worse mean than the other groups. It has been reported that pain in OA has a floating characteristic.\(^{29}\) The WOMAC sensitive to show any association between pain and frailty might have been low, because it refers to pain only in the last 72 hours. Because the OA pain is related to functional changes, fatigue, mood swings, worse quality of life, reduced independence, and worse perception of health,\(^{47-49}\) it is believed that studies assessing OA pain and frailty in a more detailed way might bring more relevant information for the clinical practice.

The duration of symptoms and radiographic changes, both in the elderly with knee OA and in those with hip OA, showed no significant differences between the frailty groups. Radiographic changes in the elderly with OA do not show, or have a weak association with, functional aspects.\(^{50}\) The
The present study assessed whether the radiographic changes were associated with frailty, but that association could not be evidenced. However, the duration of symptoms, although not yet well-defined in the literature, seems to be significant for the functional changes. In addition, it is believed that the duration of symptoms to be also significant for frailty, and, although this study shows the opposite, another study with similar numbers of elderly with initial and older OA symptoms can better clarify that aspect.

**CONCLUSION**

Of the most relevant results, the following stand out: the elderly with OA and frailty in this sample use a greater number of medications, are more obese and depressed, and have worse fall-related self-efficacy and physical function. Although this study found no significant difference among the groups regarding pain and symptom duration, such aspects should be further assessed in future studies.
CONCLUSÃO

Dentre os resultados mais relevantes, destaca-se que os idosos desta amostra com OA e fragilidade usam maior número de medicamentos, são mais obeses, mais deprimidos, têm pior autoeficácia para quedas e pior função física. Embora o estudo não tenha encontrado diferenças significativas entre os grupos no que se refere à dore e ao tempo de sintomas, acredita-se que tais aspectos mereçam atenção em pesquisas futurars.

REFERENCES

Validity and reliability of the Portuguese version of the American Shoulder and Elbow Surgeons Standardized Shoulder Assessment Form

Auristela Duarte de Lima Moser¹, Luiz Alberto Manfre Knaut², Talita Gnoato Zott², Karoleen Oswald Scharan⁴

ABSTRACT

Introduction: The American Shoulder and Elbow Surgeons Standardized Shoulder Assessment Form (ASES), often used in research in Brazil, although translated and adapted to the Portuguese language, had not had its validity and reliability tested yet. Objective: To assess the validity, reliability, and internal consistency of the ASES-PT for shoulder dysfunction.

Materials and methods: Fifty individuals (26 women; mean age, 39 ± 13 years) participated in the validity assessment, and 38 (19 women; mean age, 37 ± 13 years old) in the reliability assessment, all having shoulder dysfunction. The participants completed the 36-Item Short-Form Health Survey (SF-36), the Disabilities of the Arm, Shoulder and Hand Questionnaire (DASH), and the ASES-PT on two occasions with an interval of seven days. The convergent validity was assessed by use of the Spearman’s rank correlation coefficient (ρ), and the analysis of the intrarater reliability used the intraclass correlation coefficient (ICC). The internal consistency was assessed by using Cronbach’s alpha. Results: The ASES-PT scores correlated with the DASH scores (ρ = −0.69, P = 0.000) and with the “physical functioning” (ρ = 0.50, P = 0.000), “role limitation due to physical health” (ρ = 0.43, P = 0.002) and “bodily pain” domains (ρ = 0.60, P = 0.000) of the SF-36. The intrarater reliability of the ASES-PT proved to be adequate (ICC = 0.75, P = 0.000). The internal consistency (0.794) was satisfactory.

Conclusion: The validity and reliability study of the ASES-PT supports its use for assessing shoulder dysfunction.

Keywords: shoulder pain, shoulder joint, reproducibility of results, test validity, questionnaires.

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INTRODUCTION

Shoulder pain is present in 14%–50% of the population.¹⁻³ It is estimated that two out of three individuals will have at least one episode of neck or shoulder pain during their lives.³ In addition, the incidence of shoulder pain is of eight new cases per year for every 100 workers of the industry and service sectors.³ Frequently, that pain and the reduced shoulder mobility have a negative effect on the patient’s functional abilities, occupational activities, and quality of life.⁴

To assess and quantify the impact of the musculoskeletal changes on people’s lives, functional assessments by use of questionnaires can be performed during treatment. Several questionnaires have been developed for evaluating the function of the upper limbs.³ Of those questionnaires, the American Shoulder and Elbow Surgeons Standardized Shoulder Assessment Form (ASES) stands out because it was designed for patients with any shoulder dysfunction and specifically for that joint,³ in addition to being widely cited in the literature.⁹,¹⁰ According to Brazilian studies, the ASES has been applied
Validity and reliability of the Portuguese version of the American Shoulder and Elbow Surgeons Standardized Shoulder Assessment Form

since 1988. However, its translation and cultural adaptation for Brazilian Portuguese through the appropriate methodological process was only established in 2010.

The original ASES has been elaborated by the Research Committee of the American Shoulder and Elbow Surgeons and comprises a physician assessment section and a patient self-report section. However, only the patient self-report section contributes to its final score. That section is composed by an item concerning pain, whose severity is assessed by use of a visual analogue scale (VAS) that ranges from “no pain at all” to “pain as bad as it can be”, and 10 items concerning function, assessed by use of a four-category Likert scale. The score of each section corresponds to 50% of the final test score, which can range from 0 (no functionality) to 100 (normal function).

However, to ratify its use, the Portuguese version of the ASES (ASES-PT) should be investigated regarding its following characteristics: validity (i.e., the instrument’s capacity to measure that which it is intended to measure); reliability (i.e., the instrument’s capacity to be stable and reproducible); and internal consistency (i.e., the capacity of yielding consistent results in repeated measurements).

Thus, this study aimed at assessing the validity, reliability, and internal consistency of the ASES-PT in individuals with shoulder pain.

MATERIALS AND METHODS

Participants

Fifty individuals (26 women; mean age, 39 ± 13 years) were recruited from the school-clinics of the Pontifícia Universidade Católica do Paraná (PUCPR) for assessing the validity of the ASES-PT. All participants met the following criteria: 1) minimum age of 18 years; 2) clinical diagnosis of shoulder dysfunction; 3) neither neurological nor any musculoskeletal disease other than shoulder dysfunction; 4) no cognitive change; and 5) ability to read, understand, and complete the questionnaire studied. For assessing reliability, the sample comprised 38 individuals (19 women; mean age, 37 ± 13 years), due to sample loss of 12 individuals, who did not return to the second assessment. This study has been approved by the Ethics Committee in Research with Human Beings of the institution, according to the protocol number 5.257. All participants provided written informed consent.

Validity

To test the convergent validity, the scores obtained with the ASES-PT were correlated with those obtained with the Portuguese versions of the Disabilities of the Arm, Shoulder and Hand Questionnaire (DASH) and the 36-Item Short-Form Health Survey (SF-36), whose validity and reliability have already been shown. The DASH is a questionnaire comprising 30 questions aimed at measuring the symptoms and physical disabilities related to the upper limbs. The Portuguese version of the SF-36 is a questionnaire with 36 questions regarding the patient’s general quality of life, and covering eight domains (i.e., physical functioning, role limitation due to physical health, bodily pain, general health perceptions, vitality, social functioning, role limitation due to emotional problems, and mental health).

Validity was assessed by use of the Spearman’s rank correlation coefficient (ρ), which can range from +1 to −1. A +1 correlation indicates the existence of a perfect positive linear relationship between the variables, while a −1 correlation indicates the existence of a perfect negative linear relationship between the variables. A correlation is considered strong when ρ is greater than 0.60 or lower than −0.60; the correlation is moderate when that coefficient is between 0.30 and 0.60 or −0.30 and −0.60; and it is weak when that coefficient is between −0.30 and 0.30.

Reliability

Reliability was tested by use of the intrarater reliability (i.e., test-retest reliability), with intraclass correlation coefficient 1,1 (ICC) and 95% confidence interval. The ICC measures the agreement between the variables studied, and ranges from 0 (no agreement) to 1 (total agreement). Reliability is adequate if ICC is greater than 0.70. Because it is a self-administered questionnaire, its interrater reliability was not assessed.

Internal consistency

Internal consistency, assessed by use of the Cronbach’s alpha, was used to identify to what extent the different items of the questionnaire were associated between themselves. The total values of each item were calculated, as was the alpha’s variation when some items were eliminated. The statistical analyses were performed with the SPSS software, version 10.0 for Windows (SPSS, Chicago, USA).
Experimental procedure
To assess validity, each participant completed the ASES-PT, DASH, and SF-36 questionnaires, randomly applied. Then, the participant was invited to return to the school-clinics after seven days to conclude the reliability assessment,19 without undergoing any physical therapeutic intervention during that period. Data from the first and second ASES-PT completion were used to assess internal consistency. During the assessments, the participants were always accompanied by one of the examiners.

RESULTS
The scores of the ASES-PT showed correlation with the scores of the Portuguese version of the DASH ($\rho = -0.69; P = 0.000$) and with the following SF-36 domains: “physical functioning” ($\rho = 0.50; P = 0.000$), “role limitation due to physical health” ($\rho = 0.43; P = 0.002$), and “bodily pain” ($\rho = 0.60; P = 0.000$). With the other SF-36 domains, the correlations with the ASES-PT were weak and non-significant ($\rho \leq 0.35$; Table 1).

In the first assessment with the ASES-PT, the mean of the scores was 60.1 ± 21.1, while, in the second assessment, that mean was 63.4 ± 20.7. The intrarater reliability of the ASES-PT proved to be adequate, as shown in Table 2 (ICC = 0.75; $P = 0.000$).

Table 1
Correlation of the ASES-PT scale with the DASH scale and the SF-36 domains (n = 50)

<table>
<thead>
<tr>
<th>DASH</th>
<th>ASES-PT</th>
<th>$\rho$</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>PF</td>
<td></td>
<td>0.50</td>
<td>0.000</td>
</tr>
<tr>
<td>LPH</td>
<td></td>
<td>0.43</td>
<td>0.002</td>
</tr>
<tr>
<td>Pain</td>
<td></td>
<td>0.60</td>
<td>0.000</td>
</tr>
<tr>
<td>GHP</td>
<td></td>
<td>0.22</td>
<td>0.134</td>
</tr>
<tr>
<td>VIT</td>
<td></td>
<td>0.25</td>
<td>0.077</td>
</tr>
<tr>
<td>SF</td>
<td></td>
<td>-0.08</td>
<td>0.601</td>
</tr>
<tr>
<td>LEP</td>
<td></td>
<td>0.11</td>
<td>0.450</td>
</tr>
<tr>
<td>MH</td>
<td></td>
<td>-0.04</td>
<td>0.765</td>
</tr>
</tbody>
</table>

Table 2
Intrarater reliability of the ASES-PT and DASH scales and of the SF-36 domains (n = 38)

<table>
<thead>
<tr>
<th>Scale/domain</th>
<th>ICC (95% CI)</th>
<th>F (37,38)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASES</td>
<td>0.75 (0.57-0.86)</td>
<td>7.031</td>
<td>0.000</td>
</tr>
<tr>
<td>DASH</td>
<td>0.86 (0.75-0.93)</td>
<td>13.671</td>
<td>0.000</td>
</tr>
<tr>
<td>SF-36</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PF</td>
<td>0.60 (0.36-0.77)</td>
<td>4.114</td>
<td>0.000</td>
</tr>
<tr>
<td>LPH</td>
<td>0.57 (0.32-0.75)</td>
<td>3.744</td>
<td>0.000</td>
</tr>
<tr>
<td>Pain</td>
<td>0.57 (0.32-0.75)</td>
<td>3.713</td>
<td>0.000</td>
</tr>
<tr>
<td>GHP</td>
<td>0.36 (0.10-0.74)</td>
<td>3.623</td>
<td>0.000</td>
</tr>
<tr>
<td>VIT</td>
<td>0.67 (0.46-0.81)</td>
<td>5.208</td>
<td>0.000</td>
</tr>
<tr>
<td>SF</td>
<td>0.71 (0.52-0.84)</td>
<td>6.092</td>
<td>0.000</td>
</tr>
<tr>
<td>LEP</td>
<td>0.54 (0.27-0.73)</td>
<td>3.357</td>
<td>0.000</td>
</tr>
<tr>
<td>MH</td>
<td>0.76 (0.59-0.86)</td>
<td>7.492</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Table 3
Internal consistency of the ASES-PT scale, values of each item and Cronbach’s alpha (n = 38)

<table>
<thead>
<tr>
<th>Pain Q1 R</th>
<th>0.795</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1 Q1</td>
<td>0.788</td>
</tr>
<tr>
<td>F2 Q1</td>
<td>0.794</td>
</tr>
<tr>
<td>F3 Q1</td>
<td>0.792</td>
</tr>
<tr>
<td>F4 Q1</td>
<td>0.793</td>
</tr>
<tr>
<td>F5 Q1</td>
<td>0.793</td>
</tr>
<tr>
<td>F6 Q1</td>
<td>0.792</td>
</tr>
<tr>
<td>F7 Q1</td>
<td>0.790</td>
</tr>
<tr>
<td>F8 Q1</td>
<td>0.769</td>
</tr>
<tr>
<td>F9 Q1</td>
<td>0.797</td>
</tr>
<tr>
<td>F10 Q1</td>
<td>0.768</td>
</tr>
<tr>
<td>Pain Q2 R</td>
<td>0.786</td>
</tr>
<tr>
<td>F1 Q2</td>
<td>0.793</td>
</tr>
<tr>
<td>F2 Q2</td>
<td>0.794</td>
</tr>
<tr>
<td>F3 Q2</td>
<td>0.793</td>
</tr>
<tr>
<td>F4 Q2</td>
<td>0.790</td>
</tr>
<tr>
<td>F5 Q2</td>
<td>0.794</td>
</tr>
<tr>
<td>F6 Q2</td>
<td>0.790</td>
</tr>
<tr>
<td>F7 Q2</td>
<td>0.794</td>
</tr>
<tr>
<td>F8 Q2</td>
<td>0.774</td>
</tr>
<tr>
<td>F9 Q2</td>
<td>0.792</td>
</tr>
<tr>
<td>F10 Q2</td>
<td>0.787</td>
</tr>
</tbody>
</table>

Cronbach’s alpha 0.794

PF: physical functioning; LPH: role limitation due to physical health; GHP: general health perceptions; VIT: vitality; SF: social functioning; LEP: role limitation due to emotional problems; MH: mental health.
The results of the internal consistency of the instrument have shown good reliability indices for all items, with values greater than 0.70 and Cronbach’s alpha of 0.794 (Table 3).

DISCUSSION

Measuring instruments are used during rehabilitation programs aimed at the following: obtaining initial information about the patient; elaborating the patient’s treatment; monitoring possible changes in symptoms; and assessing the efficacy of the therapeutic procedures used. Despite the existence of its Portuguese version,\textsuperscript{13} the ASES-PT had not undergone validation and reliability investigation.

In the present study, the convergent validity of the ASES-PT was demonstrated by use of its correlation with a specific instrument for the upper limbs (i.e., DASH) and a generic instrument (i.e., the SF-36 physical health domains).

The fact that the DASH specifically assesses the symptoms and physical disabilities of the upper limbs justifies the greater correlation of the ASES-PT with that questionnaire. The results are similar to those obtained with the English version of the ASES, which showed a strong correlation when compared with a questionnaire specific for shoulder pain and function (that is, the University of Pennsylvania Shoulder Score – Penn), \((r = 0.78; P = 0.01)\).\textsuperscript{9} In addition, strong correlations between questionnaires specific for the shoulders have already been reported several times in the literature.\textsuperscript{9,20–22}

Similarly, the moderate and weak correlations between ASES-PT and the SF-36 domains observed in this study repeat the results obtained with the original version of the ASES, in which moderate correlation of the domains “physical functioning” \((r = 0.41; P = 0.001)\) and “role limitation due to physical health” \((r = 0.33; P = 0.008)\) was observed with the mean score of the physical components \((r = 0.40; P = 0.001)\), in addition to the weak correlation of the “role limitation due to emotional problems” \((r = 0.24; P = 0.21)\) and “mental health” \((r = 0.05; P = 0.70)\) observed with the mean score of the mental components \((r = 0.15; P = 0.25)\).\textsuperscript{9}

Moderate correlations between different shoulder-specific questionnaires and the SF-36 physical health domains have already been reported in previous studies.\textsuperscript{9,20} This can be explained by the fact that their items did not contemplate exclusively the activities of the upper limbs. The weak and non-significant correlations of the ASES-PT with the SF-36 emotional and mental components result from the fact that such measures assess different constructs.\textsuperscript{9}

In the present study, the reliability of the ASES-PT proved to be adequate, as already observed with its English version.\textsuperscript{9,22} However, the ICC of the Portuguese version was slightly lower than that of the English version (that is, 0.75 and 0.84, respectively). A possible explanation for that difference can be the different time intervals between the first and second application of the questionnaire. While the second assessment was applied one to three days after the first for the English version, for the Portuguese version, that interval was of seven days. Thus, a clinical change is more likely to have occurred in the participants between the assessments of the ASES-PT. However, it is worth noting that there is no consensus in the literature regarding the ideal time interval between the assessments to analyze reliability. While long time intervals can be influenced by changes in the patients’ clinical findings, short time intervals can be influenced by memory biases.

Regarding internal consistency, the scale showed good indices for all the domains of the instrument, with values over 0.70, considered satisfactory according to the literature.\textsuperscript{9,24,25} The overall reliability index of the instrument was greater than when calculated with the suppression of any item.

Even having assessed the validity, reliability, and internal consistency of the ASES-PT, its responsiveness should also be assessed, which has already been initiated by the authors of the present study to better support its use.

Because the use of questionnaires has been part of the health care routine, a careful analysis of their applicability in the various settings of the therapeutic practices is increasingly important.\textsuperscript{26} The process of assessing the psychometric properties, which can promote an increase in the resolution potential of therapeutic practices aimed at with this study, is part of that analysis.

CONCLUSION

The ASES-PT is a questionnaire of rapid application, containing clear, objective questions written in a simple language, related to daily activities. In addition, the results of this study have shown the validity, reliability, and internal consistency of the ASES-PT, indicating its adequacy for assessing shoulder function in the clinical and research practice.
DISCUSSÃO

Instrumentos de medida são utilizados durante programas de reabilitação com o objetivo de obter informações iniciais sobre o paciente, elaborar o tratamento e monitorar possíveis alterações de sintomas, além de avaliar a eficácia dos procedimentos terapêuticos empregados. Embora já apresentasse versão em português, até o presente estudo o ASES-PT não havia passado por um processo de validação e de investigação de confiabilidade.

No presente estudo, a validade convergente do ASES-PT foi demonstrada por meio de correlação com um instrumento específico para membros superiores (i.e., DASH) e com um instrumento genérico (i.e., domínios de aspecto físico do SF-36).

O fato de o DASH avaliar especificamente os sintomas e as incapacidades físicas dos membros superiores justifica a maior correlação do ASES-PT com esse questionário. Os resultados são semelhantes aos obtidos pela versão em inglês do ASES, que apresentou correlação forte ao ser comparado com um questionário específico para dor e função do ombro (i.e., The University of Pennsylvania Shoulder Score – Penn), \( r = 0.78; P = 0.01 \). Além disso, correlações fortes entre questionários específicos para ombro já foram diversas vezes apresentadas na literatura.

Da mesma forma, as correlações moderadas e fracas entre ASES-PT e os domínios do SF-36, observadas neste estudo, repetem os resultados com a versão original do ASES, na qual houve correlação moderada com o domínio “capacidade funcional” \( r = 0.41; P = 0.001 \) e “limitação por aspectos físicos” \( r = 0.33; P = 0.008 \) e o escore médio dos componentes físicos \( r = 0.40; P = 0.001 \), além de fraca correlação com “limitação por aspectos emocionais” \( r = 0.24; P = 0.21 \), “saúde mental” \( r = 0.05; P = 0.70 \) e o escore médio dos componentes mentais \( r = 0.15; P = 0.25 \).

Correlações moderadas entre diferentes questionários específicos para o ombro e os domínios de aspectos físicos do SF-36 já foram apresentadas em estudos anteriores. Isto pode ser explicado pelo fato de seus itens não contemplarem apenas atividades com os membros superiores. As correlações fracas e não significativas do ASES-PT com os componentes emocional e mental do SF-36 ocorrem em razão de tais medidas avaliarem diferentes construtos.

No presente estudo, a confiabilidade do ASES-PT demonstrou-se adequada, assim como já havia sido observada em sua versão em inglês. No entanto, o CCI da versão em português foi um pouco inferior ao da versão em inglês (i.e., 0.75 e 0.84, respectivamente). Uma possível explicação para essa diferença pode ser o intervalo entre a primeira e a segunda aplicação do questionário. Enquanto a segunda avaliação foi aplicada no intervalo de 1–3 dias após a primeira, para a versão em português o intervalo foi de sete dias. Assim, é mais provável que tenha ocorrido uma alteração clínica no participante entre as avaliações no estudo do ASES-PT. No entanto, é importante ressaltar que não há consenso na literatura quanto ao intervalo ideal para investigar a confiabilidade. Enquanto intervalos longos podem ser influenciados por mudanças no quadro clínico do paciente, intervalos curtos podem sofrer vieses de memória.

No que se refere à consistência interna, a escala demonstrou bons índices de fidedignidade para todos os domínios do instrumento, com valores superiores a 0,70, considerados satisfatórios de acordo com a literatura. O índice de confiabilidade do instrumento como um todo foi maior que quando calculado com supressão de quaisquer dos itens.

Mesmo tendo sua validade, confiabilidade e consistência interna avaliados, considera-se importante o estudo da responsividade, já iniciado pelos autores do presente estudo a fim de melhor embasar sua utilização.

Como a utilização de questionários tem sido parte integrante das rotinas de atendimento, é cada vez mais importante uma análise criteriosa de sua aplicabilidade nos vários cenários de práticas terapêuticas. Fazem parte dessa análise o processo de avaliação das propriedades psicométricas, que pode promover o aumento do potencial de resolubilidade das práticas terapêuticas, o que se espera com a divulgação deste estudo.

CONCLUSÃO

O ASES-PT é um questionário de rápida aplicação, formado por perguntas claras, objetivas e com linguagem simples, relacionadas às atividades corriqueiras que fazem parte do cotidiano da maioria das pessoas. Além disso, os resultados deste estudo demonstram sua validade, confiabilidade e consistência interna, indicando que é um instrumento adequado para avaliar a função do ombro nos meios clínicos e de pesquisa.

REFERENCES

Antinucleosome antibodies and primary antiphospholipid syndrome: an observational study
Alexandre Wagner Silva de Souza¹, Silene Peres Keusseyan², Neusa Pereira da Silva³, Emilia Inoue Sato⁴, Luis Eduardo Coelho Andrade⁵

ABSTRACT

Objective: To study the association of anti-nucleosome (anti-NCS) antibodies in primary antiphospholipid syndrome (APS) and the development of systemic lupus erythematosus (SLE) during follow-up. Materials and methods: Thirty-six women with primary APS were evaluated prospectively for clinical features of systemic autoimmune diseases and for the presence of antiphospholipid antibodies, antinuclear antibodies and anti-NCS/chromatin antibodies. Results: After a mean follow-up period of 45.7 months, anti-NCS/chromatin antibodies were detected in only one patient (2.8%), who developed features of SLE including polyarthritis, lymphopenia, optic neuritis, multiple sclerosis-like lesions, and an autoantibody profile suggestive of SLE. Conclusion: The frequency of anti-NCS/chromatin antibodies in primary APS patients is very low, and they may be associated with the development of SLE manifestations.

Keywords: antiphospholipid syndrome, systemic lupus erythematosus, anticardiolipin antibodies, lupus coagulation inhibitor nucleosomes.

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INTRODUCTION

The antiphospholipid syndrome (APS) is characterized by thrombotic manifestations and/or obstetrical morbidity in the presence of antiphospholipid antibodies (APL). The standard APL assays recommended by the latest guidelines on APS are the lupus anticoagulant (LAC) and enzyme immunoassays for antibodies against β₂ glycoprotein I (anti-β₂ GPI) and against cardiolipin (aCL) in the presence of β₂ GPI. APS can occur as a primary disease or associated with some other systemic autoimmune disease, predominantly systemic lupus erythematosus (SLE).¹,²

SLE is characterized by a wide range of circulating autoantibodies, several of which are directed against nuclear antigens.³ In particular, chromatin antigens appear to be a preferential target of autoantibodies in SLE. Nucleosome is the unit of chromatin and consists of 146 base pairs of DNA wrapped around a protein core. The protein core is an octamer consisting of two molecules of each of the histones H2A, H2B, H3, and H4.⁴ The frequency of autoantibodies against nucleosome (anti-NCS) and H1 stripped chromatin (anti-chromatin) in SLE varies from 50%–100% and the specificity for SLE diagnosis has been reported from 90%–99%.⁵-¹⁰ Anti-NCS/chromatin antibodies have been associated with active glomerulonephritis in SLE patients.⁶-¹⁰

Although considered specific for SLE, anti-NCS/chromatin antibodies have been reported in other autoimmune conditions such as systemic sclerosis, Sjögren’s syndrome,
mixed connective tissue disease, and type 1 autoimmune hepatitis.6,8,10,16,17 There is some controversy in the literature and some authors believe that the finding of positive reactivity in non-lupus patients is due to heterogeneity in NCS/chromatin preparations and other reagents used in enzyme-linked immunosorbent assay (ELISA) tests.6,18–20

Recently, different groups have reported on anti-NCS/chromatin antibodies in primary APS.9,21,22 However, the prevalence of anti-NCS antibodies is wide among primary APS patients, ranging from 7%–77%; this may be related either to methodological issues in the detection of anti-NCS/chromatin antibodies or to the inclusion of patients with lupus-like syndrome. Many of the anti-NCS/chromatin positive patients with primary APS were found to subsequently develop SLE.21,22 Thus, it is not established whether primary APS patients carrying anti-NCS/chromatin antibodies are in fact SLE patients with incipient disease. Therefore, we set to develop an observational prospective study to evaluate the frequency of anti-NCS/chromatin antibodies in patients with primary APS and the development of defined SLE or isolated SLE traits.

MATERIALS AND METHODS

Sampling, recruitment and data collection

Thirty-six women meeting the Sapporo criteria for primary APS23 underwent clinical evaluation for manifestations of APS and other autoimmune diseases. Peripheral blood was obtained at study entry for determination of LAC and aCL, anti-β2 GPI and anti-NCS/chromatin antibodies. Patients were prospectively evaluated for a mean of 45.7 ± 9.6 months (range 13–56) with special attention for evidence of systemic autoimmune disease or the establishment of SLE according to the American College of Rheumatology updated criteria for SLE classification.24 Exclusion criteria at study entry were the presence of systemic autoimmune disease other than APS, chronic infection, malignancy, and age below 18 years. All participants signed an Informed Consent approved by the institutional Ethics Committee.

Antibody assays

Anti-cardiolipin antibodies were detected using an in-house standard ELISA technique.25 Briefly, ELISA plates (NUNC – Thermo Fisher Scientific Inc, Roskilde, Denmark) were coated (50 µL per well) with bovine cardiolipin (50 µg/mL) (Sigma Aldrich Inc, St Louis, USA) overnight at 4 °C. After blocking with 100 µL of 10% adult bovine serum (ABS) in phosphate buffered saline pH 7.4 (PBS) for 2 hours at room temperature (RT), 50 µL of patient serum diluted 1:50 in 10% ABS in PBS were added to duplicate wells and incubated overnight at 4 °C. Plates were then washed three times with PBS and wells received alternatively 50 µL anti-IgM or anti-IgG peroxidase-conjugates (Calbiochem, La Jolla, CA, USA) diluted 1:4,000 and 1:5,000 in PBS, respectively, and then were incubated for 90 minutes at RT. After two additional washing steps as before, 50 µL p-nitrophenyl phosphate disodium in diethanolamine buffer (pH 9.8) were added to the wells.

The optic density (OD) was determined by spectrophotometry at 450 nm wave length at 15-minute intervals until the standard positive sample reached an OD between 1,000 and 1,200. The calibration curve was based on international APL standards (Louisville APL Diagnostics Inc, Doraville, GA, USA). All samples were processed in duplicate. Results were expressed in GPL and MPL units for IgG and IgM aCL, respectively, and positive values were considered when above 20 GPL or 20 MPL units. LAC was detected using activated partial thromboplastin time (APTT – Diagnostica Stago, France) and diluted Russell’s viper venom time (dRVVT – Trinity Biotech, Wiclow, Ireland, UK) according to international guidelines.26 Serum IgG and IgM anti-β2 GPI were detected by ELISA (The Binding Site, Birmingham, UK), according to the manufacturer’s instructions with cutoff values of 10 U/mL for IgM and 20 U/mL for IgG.

Anti-NCS/chromatin antibodies were detected by ELISA (INOVA Diagnostics, San Diego, CA, USA), according to the manufacturer’s instructions. Antinuclear antibodies (ANA) were determined by indirect immunofluorescence on HEp-2 cells (Bion, Des Plaines, IL, USA) at a screening dilution of 1:80, according to standard protocol.27,28 Anti-double stranded DNA (dsDNA) tests were performed by indirect immunofluorescence using Crithidia luciliae as substrate; anti-extractable nuclear antigens (ENA) were detected by double immunodiffusion technique and included anti-SSA/Ro, anti-SSB/La, anti-Sm, and anti-RNP antibodies. The Western blot technique was used to detect at least one band of three ribosomal P proteins P0 (38 kD), P1 (19 kD), and P2 (17 kD).

Statistical analysis

Statistical analysis was carried out with SPSS 10.0 for Windows, Chicago, USA. Results were expressed as total number and percentage for categorical data and as mean and standard deviation (SD) for continuous variables.
RESULTS

Features of primary APS patients

Thirty-six women with primary APS with a mean age of 38.4 ± 11.8 years participated in this study. Fifteen (42%) were Caucasian-descendants and 21 (58%) were African-descendants. Previous arterial thrombosis episodes occurred in 13 patients (36.1%) being stroke the most frequent manifestation, occurring in 11 (84.6%) of these cases. Previous venous thrombotic episodes occurred in 17 patients (47.2%) mostly in the lower limbs (13 patients, 76.4%). Seventeen patients (47.2%) presented pregnancy morbidity, which was the only APS manifestation in six of them (Table 1). Along the follow-up period, aCL was present in 31 patients (86.1%), IgG aCL was found in 28 (77.8%), and IgM aCL in eight patients (22.2%). Eleven patients (30.6%) had a positive LAC test. Anti-β2 GPI antibodies were detected in eight patients (22.2%): IgM anti-β2 GPI in five (13.9%) and IgG anti-β2 GPI in six patients (16.7%). The concomitant presence of the three APS-associated autoantibodies (aCL, LAC, and anti-β2 GPI antibodies) occurred in six cases (16.7%) (Table 1). ANA was detected in 12 patients (33.3%) and the most frequent ANA pattern was the nuclear speckled pattern, that occurred at 1/320 titer in eight patients. Other ANA patterns, such as nuclear homogeneous (two cases), nuclear envelope (one case), and discrete cytoplasm speckles (one case), were also observed.

Anti-NCS/chromatin antibodies and disease follow-up in primary APS patients

Anti-NCS/chromatin antibodies were positive in only one patient (2.8%) with primary APS (Table 1). Considering that the recommended cut-off for the test is 20 IU/mL and strongly positive reactivity is set above 60 IU/mL, it is relevant to mention that this patient presented 147 IU/mL (Figure 1). Along the follow-up period (45.7 ± 9.6 months) this patient with reactivity for the anti-NCS/chromatin assay developed manifestations of SLE such as lymphopenia, polyarthitis, and neurological involvement characterized by optic neuritis and white matter demyelinating lesions resembling multiple sclerosis on brain MRI. She also developed an autoantibody profile compatible with SLE, including a 1:1280 antinuclear test (Hep-2 ANA) with homogeneous pattern, anti-dsDNA 1/10, anti-SS-A/Ro and anti-ribosomal P antibodies. This patient had a non-reactive test for anti-aquaporin 4 antibody. Among patients with no reactivity for anti-NCS/chromatin antibodies, two developed rheumatoid-like polyarthritis and had non-reactive tests for rheumatoid factor and cyclic citrullinated peptide antibody. One of them was treated with corticosteroids and methotrexate and another one with methotrexate and leflunomide. One death occurred due to myocardial infarction in one patient after a mean follow-up time of 13 months. No other patient developed symptoms and signs suggestive of systemic involvement. No other patient was reactive for anti-dsDNA and ENA.

---

**Table 1**

Clinical features and autoantibody profile in primary APS patients

<table>
<thead>
<tr>
<th>Variables</th>
<th>Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Thrombosis</strong></td>
<td>30 (83.3%)</td>
</tr>
<tr>
<td><strong>Venous manifestations</strong></td>
<td></td>
</tr>
<tr>
<td>Lower limbs</td>
<td>13</td>
</tr>
<tr>
<td>Pulmonary embolism</td>
<td>3</td>
</tr>
<tr>
<td>Intracranial venous thrombosis</td>
<td>2</td>
</tr>
<tr>
<td>Upper limbs</td>
<td>1</td>
</tr>
<tr>
<td><strong>Arterial manifestations</strong></td>
<td></td>
</tr>
<tr>
<td>Stroke</td>
<td>11</td>
</tr>
<tr>
<td>Peripheral occlusion</td>
<td>3</td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>1</td>
</tr>
<tr>
<td><strong>Pregnancy morbidity</strong></td>
<td>17 (47.2%)</td>
</tr>
<tr>
<td><strong>APS-related autoantibodies (frequency)</strong></td>
<td></td>
</tr>
<tr>
<td>LAC</td>
<td>11 (30.6%)</td>
</tr>
<tr>
<td>IgG aCL</td>
<td>28 (77.8%)</td>
</tr>
<tr>
<td>IgM aCL</td>
<td>8 (22.2%)</td>
</tr>
<tr>
<td>Anti-β2 GPI</td>
<td>8 (22.2%)</td>
</tr>
<tr>
<td>All three antiphospholipid antibodies</td>
<td>6 (16.7%)</td>
</tr>
<tr>
<td><strong>Anti-nucleosome/chromatin antibodies (frequency)</strong></td>
<td></td>
</tr>
<tr>
<td>Anti-chromatin</td>
<td>1 (2.8%)</td>
</tr>
</tbody>
</table>

**Figure 1**

Magnitude of IgG anti-NCS/chromatin antibody reactivity in primary APS patients. Levels between 20.0 IU/mL and 60.0 IU/mL are considered moderately positive and levels above 60 IU/mL, strongly positive.
DISCUSSION

The present study has demonstrated a low frequency of antibodies against chromatin components in primary APS. In fact, only one patient (2.8%) had a positive anti-NCS/chromatin test and developed SLE. The vast majority of the primary APS patients did not develop overt signs and symptoms compatible with SLE along the mean 45-month follow-up period. As an exception, however, the one patient with reactivity in the anti-NCS/chromatin assay presented lymphopenia, polyarthritis, optic neuritis, white matter demyelinating lesions suggestive of multiple sclerosis, and an autoantibody profile highly suggestive of SLE.

The presence of anti-NCS/chromatin antibodies in patients with primary APS has been subject of some controversy in the recent literature. Since anti-NCS/chromatin antibodies are considered as highly specific for SLE, the occurrence of these autoantibodies in a patient with current diagnosis of primary APS might indicate the possibility of progression towards SLE. In fact, it has been shown by one group that the prevalence of anti-NCS/chromatin antibodies in primary APS is low and other groups associated their presence in APS patients with subsequent development of SLE features.\textsuperscript{9,12} Although the development of manifestations of APS in SLE patients with APL antibodies has been reported in 50\%–70\% of cases within twenty years of follow-up,\textsuperscript{1,29} the opposite is not usually expected. Actually, a complete picture of SLE may evolve in 4\%–10\% primary APS patients, and the presence of a family history of SLE, Raynaud phenomenon, migraine, multiple sclerosis-like features, hemolytic anemia, low C3 and C4, and Coombs’ test positivity are recognized risk factors.\textsuperscript{30} The present finding suggests that anti-NCS/chromatin antibodies might be included as an additional risk factor for SLE development in patients with a diagnosis of primary APS.

The antigenic substrate used in the anti-NCS/chromatin assays is relevant to the heterogeneity in results obtained by different authors. The commercial assay used in the present study was H1-stripped chromatin devoid of non-histone proteins (mainly oligonucleosomes) derived from calf thymus. This is a widely used antigen for anti-NCS/chromatin assays and contains most nucleosome epitopes recognized by polyclonal and monoclonal anti-NCS antibodies.

The lack of standardization of serologic tests is a major problem for autoantibody determination.\textsuperscript{31} The antigens currently used for determination of anti-NCS/chromatin antibodies include H1-stripped chromatin, polynucleosomes devoid of H1, and mononucleosomes with or without H1. In addition, there is variation in the biochemical conditions for antigen purification and in the raw source used for antigenic preparation. Finally there is heterogeneity in the conditions established in the various immunoassays. This overall heterogeneity implies that the different assays do not detect exactly the same autoantibody population and this may contribute to the partially conflicting results observed in the literature.\textsuperscript{32}

In conclusion, the present study showed a low frequency of antibodies against NCS/chromatin in patients with primary APS and those autoantibodies may be associated with the development of SLE features in patients with a diagnosis of primary APS. Further multicentric and longitudinal studies should be performed to confirm this finding.

ACKNOWLEDGEMENTS

The authors thank Silvia Helena Rodrigues for doing ANA tests and Maria Teresa Costa for collecting blood samples of all participants in this study.
outra com metotrexato e leflunomida. Uma paciente morreu devido a infarto do miocárdio após um seguimento médio de 13 meses. Nenhuma outra paciente desenvolveu sintomas e sinais sugestivos de envolvimento sistêmico. Nenhuma outra paciente foi positiva para anti-dsDNA e ENA.

DISCUSSÃO

O presente estudo demonstrou baixa frequência de anticorpos contra componentes da cromatina na SAFP. De fato, apenas uma paciente (2,8%) apresentou positividade para o ensaio de anti-NCS/cromatina e desenvolveu LES. A maioria das pacientes com SAFP não desenvolveu sinais nem sintomas compatíveis com LES durante o seguimento médio de 45 meses. A única exceção, no entanto, uma paciente com positividade para o ensaio de anti-NCS/cromatina, apresentou linfopenia, poliartrite, neurite óptica, lesões desmielinizantes na substância branca sugestivas de esclerose múltipla e um perfil de anticorpos altamente sugestivo de LES.

A presença de anticorpos anti-NCS/cromatina em pacientes com SAFP tem sido motivo de controvérsia na literatura recente. Como os anticorpos anti-NCS/cromatina têm sido considerados altamente específicos para LES, a presença desses autoanticorpos em uma paciente com diagnóstico de SAFP pode indicar progressão da doença para LES. Um grupo de pesquisadores demonstrou que a prevalência de anticorpos anti-NCS/cromatina na SAFP é baixa,9 tendo outros grupos associado a presença desses anticorpos em pacientes com SAF com o desenvolvimento de características de LES.21,22 Embora o desenvolvimento de manifestações de SAF em pacientes lúpicos com anticorpos AAF tenha sido relatado em 50%–70% dos casos ao longo de 20 anos de seguimento,1,29 o oposto, em geral, não é esperado. Na verdade, um quadro completo de LES pode se desenvolver em 4%–10% dos pacientes com SAFP, com presença de história familiar de LES, fenômeno de Raynaud, enxaqueca, achados semelhantes a esclerose múltipla, anemia hemolítica, níveis baixos de C3 e C4 e teste de Coombs positivo sendo reconhecidos como fatores de risco.30 Os achados atuais sugerem que os anticorpos anti-NCS/cromatina podem ser considerados um fator de risco adicional para o desenvolvimento de LES em pacientes com SAFP.

O substrato antígenico usado nos ensaios de anticorpos anti-NCS/cromatina é importante para a heterogeneidade dos resultados obtidos por diferentes autores. O ensaio comercial utilizado no presente estudo foi a cromatina sem H1 e sem as proteínas histonas (principalmente oligonucleossomos) derivada do timo de bezerro. Trata-se de um antígeno largamente usado para ensaios de anti-NCS/cromatina, contendo a maioria dos epitéplos de nucleossomo reconhecidos pelos anticorpos anti-NCS policlonais e monoclonais.

A falta de padronização dos testes sorológicos é o problema principal para a determinação de autoanticorpos.23 Os antígenos atualmente utilizados para a determinação dos anticorpos anti-NCS/cromatina incluem cromatina sem H1, polinucleossomos sem H1 e mononucleossomos com ou sem H1. Além disso, há uma variação nas condições bioquímicas de purificação de antígenos e na fonte natural usada para a preparação de antígenos. Finalmente, as condições estabelecidas nos vários imunoensaíos são heterogêneas. Toda essa heterogeneidade implica que os diferentes ensaios não detectam exatamente a mesma população de autoanticorpos, o que pode contribuir para os resultados parcialmente conflitantes observados na literatur.32

O presente estudo mostrou uma baixa frequência de anticorpos anti-NCS/cromatina em pacientes com SAFP, e esses autoanticorpos podem estar associados ao desenvolvimento de características de LES naqueles pacientes. Estudos multicêntricos e longitudinais adicionais são necessários para confirmar tais achados.

AGRADECIMENTOS

A Silvia Helena Rodrigues por realizar os testes para ANA, e a Maria Teresa Costa por coletar as amostras de sangue de todos os participantes deste estudo.

REFERENCES

Anticorpos antinucleossomo e síndrome antifosfolipídica: estudo observacional


Association of HLA-DRB5*01 with protection against cutaneous manifestations of rheumatoid vasculitis in Brazilian patients

Wester Eidi Nishimura1, Lilian Tereza Lavras Costallat2, Sandra Regina Muchinechi Fernandes3, Roseneide Aparecida Conde4, Manoel Barros Bertolo5

ABSTRACT

Objective: To evaluate the frequency of HLA classes I and II and their association with the cutaneous manifestation of rheumatoid vasculitis (RV) in Brazilian patients. Patients and methods: During one year we selected 130 patients with rheumatoid arthritis (RA) classified according to the American College of Rheumatology, 1987. All patients underwent a clinical and laboratory questionnaire to exclude other causes of cutaneous vasculopathy (neoplasia, infections, illicit drug use, diabetes mellitus, and tobaccoism). Seventy-three patients with any risk factor for other causes of vasculopathy were excluded. Fifty-seven without risk factors for other causes of vasculopathy were included in the study, 17 with RV according to Scott and Bacon’s criteria, 1984. Demographic data, time of RA diagnosis, disease activity (DAS28), presence of rheumatoid factor, and anti-cyclic citrullinated peptide antibodies were analyzed. The HLA alleles were typed using the DNA-amplified polymerase chain reaction with low-resolution hybridization and sequence-specific primers. Results: The comparison between the 40 patients without RV and the 17 patients with RV showed an increased frequency of HLA-B*14 (Pc = 0.168) and HLA-Cw*08 (Pc = 0.084) in patients with RV and an increased frequency of HLA-DRB5*01 (Pc = 0.048) in patients without RV. Conclusion: The HLA-DRB5*01 may confer protection against that extra-articular manifestation of RA.

Keywords: rheumatoid vasculitis, HLA antigens, disease susceptibility, protection.

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INTRODUCTION

Autoimmune diseases are often associated with human leukocyte antigen (HLA) genes, which are encoded in the major histocompatibility complex. HLA molecules participate in antigen presentation and cell response playing an important role in the immune response. Their effect on T cell repertoire is striking and crucial in the pathogenesis of rheumatoid arthritis (RA), especially in a more severe form of disease, interacting with the HLA system. HLA-B8 combined/linked DR3, HLA-DRB1*04, HLA-Cw*03, and HLA-DQB1*0311,12 have been associated with rheumatoid vasculitis (RV) in populations with homogeneous racial/ethnics characteristics. We recently studied some cases of autoimmune vasculitis and HLA and could note that HLA-DR alleles may influence their clinical expression and outcome in a mixed population.13

RV is a systemic manifestation of RA that can affect many organs, but our aim was to evaluate cutaneous manifestation. We aimed to evaluate the frequency rate of HLA classes I and II as well the possibility of association with cutaneous manifestation of RV in Brazilian patients, checking their relationship with demographic factors, time of RA diagnosis, disease activity, and laboratory data in comparison with RA patients without RV or with other causes of vasculopathy, because there are no studies relating RV and HLA in mixed population.
PATIENTS AND METHODS

Patients and controls

One hundred thirty patients in treatment of RA using disease-modifying antirheumatic drugs (DMARDs) during the year of 2006, performing routine visits in the Ambulatory Service of Rheumatology at the Hospital Universitário, Universidade Estadual de Campinas (Unicamp), Campinas, SP, Brazil, were prior selected. They agreed to participate in the study after reading and signing the Consent Form approved by the Medical Ethics Committee. A clinical and laboratory questionnaire was applied for exclusion of other causes of cutaneous vasculopathy, such as neoplasia, infections, illicit drug use, diabetes mellitus, and tobaccoism.

The questionnaire was applied for all patients and 73 were excluded because of history of smoking more than 20 cigarettes/day, glucose greater than 126 mg/dL in two takes, positive serology for hepatitis B or C, illicit drug use, and/or neoplasia. Of these, four patients had episodes of deep cutaneous ulcer and digital infarcts, but this was not seen during the selection, and three patients had such clinical affections visible during the selection. According to the Scott and Bacon’s criteria,14 17 patients were diagnosed with RV due to the presence of deep cutaneous ulcers associated with digital infarcts visible at the time of selection and without clinical data or image for visceral involvement of vasculopathy. Forty patients never showed episodes of cutaneous or visceral vasculopathy during one year of study.

The patient’s age ranged from 17–78 years (46 females), comprising 32 Caucasians, self-described to be of Western and Southern European ancestries; 14 Mulattoes (Caucasian and Black admixtures); and 11 Blacks, historically mostly of African ancestries (Bantu, Benin and Senegal).15,16 RA was diagnosed in accordance to the American College of Rheumatology (ACR) criteria, 1987.17

The same clinical and laboratory questionnaire also included an analysis of records containing demographic data, such as gender, age, and race/ethnicity, as well as time of clinical diagnosis of initial RA and EULAR Disease Activity Score-28 (DAS28); and laboratory data, such as rheumatoid factor (RF), and antibodies to cyclic citrullinated peptide (anti-CCP). The calculated values of DAS28 were considered as > 5.1 for very active disease, as > 3.2 ≤ 5.1 for moderate disease activity, and as ≤ 3.2 for inactive disease.18

All 57 patients were submitted to HLA classes I and II genotyping, being statistically analyzed by the Biostatistics Service at the Research Commission of the Faculty of Medical Sciences, Unicamp, Campinas, SP, Brazil.

Genotyping

HLA-class I genotyping was performed by the polymerase chain reaction amplification (PCR) technique using sequence of specific primers (One Lambda Inc., CA, UK). HLA-class II genotyping was performed by the PCR amplification technique using sequence of specific primers (low resolution – DYNAL, Biotech Ltd., UK).

Detection of autoantibodies

Enzyme immunoassays using anti QUANTA Lite™ CCP3 IgG (INOVA) detected antibodies semiquantitatively, and the values were considered positive when > 22 IU/dL.

Detection of rheumatoid factor

RF was detected by the quantitative method of nephelometry, and the values were considered positive when > 30 IU/mL.

Statistical analysis

The frequency of alleles in patients and controls was analyzed using the Fisher’s exact test. The Mann-Whitney test or χ2 test was used for numerical variables, such as age, DAS28, gender, race/ethnicity, RF, anti-CCP and time of RA diagnosis. The statistical software used for data analysis was SPSS 17.0. The Bonferroni correction (Pc) was used for significant statistical values, after using the Fisher’s exact test. P values were considered significant when < 0.05.

RESULTS

Most patients with RV were Caucasian female patients, but the demographic showed no statistical difference about gender, age, and race/ethnicity between the two groups of patients. Regarding time of RA diagnosis there was also no statistical difference between the groups. The average time of RA diagnosis in the 17 patients with RV was 13.1 years. Since most patients with RV were positive for RF and anti-CCP, there was no statistical difference between the groups as well. Regarding DAS28, there was no statistical difference (Pc = 0.057) among patients with RV in comparison with patients without RV (Table 1).

The patients expressed HLA-A (*01/*02/*03/*11/*23 /*24/*25/*26/*29/*30/*32/*34/*66/*68/*74/*80), HLA-B (*07/*08/*13/*15/*18/*27/*35/*37/*38/*39/*40/*42/*44/ *45/*46/*47/*49/*50/*51/*52/*53/*55/*56/*57/*58/*59/81), HLA-C (*01/*02/*03/*04/*05/*06/*07/*12/*14/*15/*16/*17/*18), HLA-DRB1 (*01/*03/*04/*07/*08/*09/*10/*1
Hla-DRB3 (*01), HLA-DRB4 (*01), and HLA-DQB1(*02/*03/*04/*05/*06).

However, only HLAB*14 (P = 0.006), HLA-Cw*08 (P = 0.006), and HLA-DRB5*01 (P = 0.048) typings were statistically significant by the Fisher’s exact test. The Bonferroni correction was applied for HLA-B*14, HLA-Cw*08, and HLA-DRB5*01, which were statistically significant by the Fisher’s exact test, showing HLA-B*14 (Pc = 0.168) and HLA-Cw*08 (Pc = 0.084) in patients with RV and HLA-DRB5*01 (Pc = 0.048) in patients without RV (Table 2).

DISCUSSION

In this study, RV was observed in a higher proportion of patients (29.8%) when compared with other studies (2.1%), because our sample was small before the patients which agreed to participate in the study during one year and after excluding patients with a risk factor for other causes of cutaneous vasculopathy. The female gender was prevalent in both groups, due to RA being a more common disease in women. There was no statistical difference regarding gender, age, and race/ethnicity between the two groups. The female gender was more frequent in our patients with RV, differing from other studies, which showed a male predominance.

There is no previous data on race/ethnicity in RV, but Caucasians were prevalent in the present study in both groups, since this is the predominant race/ethnicity in the Southeastern region of Brazil, where the study took place. Regarding time of RA diagnosis, there was also no statistical difference in the groups. The average time of RA diagnosis in 17 patients with RV was 13.1 years, which proved to be similar to other studies that show the RV emergence after more than 10 years of RA. Regarding the DAS28, there was no statistical difference between patients with and without RV after the Bonferroni correction. RF and anti-CCP were positive for most patients with RV, in agreement with other studies; however, there was no statistical difference between the two groups.

The importance of detecting HLA in patients with RA in a mixed population has been demonstrated in other studies; however, until the publication of the present study, there were no articles correlating HLA and RV in patients with RA in the Brazilian population. Regarding the association between HLA and RV, articles have been previously published in homogeneous populations showing correlation with HLA-B8 combined/linked DR3, while the HLA-DRB1 molecule is more evident in most studies, possibly because it is expressed at a level five times higher than its paralogues HLA-DRB3, DRB4 and DRB5.

This study demonstrated an increased frequency of HLA-B*14 and HLA-Cw*08 alleles in patients with RV, which was not previously reported. However, this increased frequency showed statistical significance only by the Fisher’s exact test, which was not observed by the Bonferroni correction. The increased frequency of HLA-DRB5*01 appeared to provide protection against RV in our study, but was not observed in any other studies, with statistical significance both by the Fisher’s exact test and the Bonferroni correction.
A study was published correlating the HLA-DRB5 null with worsening of autoimmune disease,\textsuperscript{28} which was not observed in our study, where the *01 allele was amplified and had associated protection. The presence of HLA-DRB5 is linked with allelic variants of HLA-DRB1, otherwise it is omitted.\textsuperscript{29}

We concluded that, although the Brazilian population is usually mixed, the findings are not similar to those found in homogeneous populations with RV, the HLA-DRB1 being the primary susceptibility gene for protection. RV was more evident in female patients, Caucasian race, RF, and anti-CCP positive, but the data were not statistically significant. HLA-B*14 and HLA-Cw*08 were not statistically significant regarding susceptibility to RV. Considering that HLA-DRB5*01 is significantly less frequent in patients with RV, it may confer protection against this extra-articular manifestation of RA. The presence of HLA-DRB5 is due to the allelic variants of HLA-DRB1, which may behave differently in a mixed population with a higher allelic variance than in homogeneous populations. A multicenter study in the Southeast Brazilian region will enable to increase the sample of patients and thus observe the expression and protection of HLA found.
Este estudo demonstrou uma frequência aumentada de alelos HLA-B*14 e HLA-Cw*08 em pacientes com VR, o que não foi previamente relatado. Entretanto, apenas o teste exato de Fisher mostrou significância estatística, que não foi observada pela correção de Bonferroni. Em nosso estudo, a frequência aumentada de HLA-DRB5*01 pareceu oferecer proteção contra VR, com significância estatística tanto pelo teste exato de Fisher quanto pela correção de Bonferroni. Isso não foi observado em nenhum outro estudo.

Um estudo correlacionou o HLA-DRB5 null com o agravamento de doença autoimune,28 o que não foi observado em nosso estudo, onde o alelo *01 foi amplificado e associou proteção. A presença de HLA-DRB5 está ligada a variantes alélicas de HLA-DRB1, sendo, do contrário, omitida.29

Em conclusão, como a população brasileira em geral é mista, os achados não são semelhantes àqueles de populações homogêneas com VR, sendo o HLA-DRB1 o gene de suscetibilidade primária para proteção. A VR foi mais evidente em mulheres caucasianas, positivas para FR e anti-CCP, mas os resultados não foram consistentes tanto pelo teste exato de Fisher quanto pela correção de Bonferroni. Isso não foi observado em nenhum outro estudo.

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Em conclusão, como a população brasileira em geral é mista, os achados não são semelhantes àqueles de populações homogêneas com VR, sendo o HLA-DRB1 o gene de suscetibilidade primária para proteção. A VR foi mais evidente em mulheres caucasianas, positivas para FR e anti-CCP, mas os resultados não foram consistentes tanto pelo teste exato de Fisher quanto pela correção de Bonferroni. Isso não foi observado em nenhum outro estudo.

**REFERÊNCIAS**


Low prevalence of renal, cardiac, pulmonary, and neurological extra-articular clinical manifestations in spondyloarthritis: analysis of the Brazilian Registry of Spondyloarthritis


ABSTRACT

Objective: To describe the extra-articular manifestations (cardiac, renal, pulmonary, and neurological), usually not related to spondyloarthritis (SpA), in a large cohort of Brazilian patients. Materials and methods: This retrospective study analyzed 1,472 patients diagnosed with SpA and cared for at 29 health care centers distributed in the five major geographic regions in the country, participating in the Brazilian Registry of Spondyloarthritis (BRS). All patients were assessed for the prevalence of major extra-articular manifestations (cardiac, renal, pulmonary, and neurological), classified according to the diagnosis [ankylosing spondylitis (AS), psoriatic arthritis (PsA), reactive arthritis (ReA), arthritis associated with inflammatory bowel disease (IBD), undifferentiated spondyloarthritis (uSpA), and juvenile SpA], and according to the clinical presentation (axial, peripheral, mixed, and enthesitis). Results: Of the patients with SpA assessed, 963 had AS, 271 PsA, 49 ReA, 48 arthritis associated with IBD, 98 uSpA, and 43 juvenile SpA. Cardiac involvement was reported in 44 patients (3.0%), pulmonary involvement in 19 (1.3%), renal involvement in 17 (1.2%), and neurological involvement in 13 patients (0.9%). Most patients with visceral involvement had AS or PsA, and the mixed (axial + peripheral) and/or predominantly axial clinical form. Conclusion: Cardiac, renal, pulmonary, and neurological extra-articular manifestations are quite infrequent in SpA, ranging from 0.9% to 3% in this large Brazilian cohort, and affected predominantly patients with AS and PsA.

Palavras-chave: spondylitis, neurology, cardiology, pulmonary medicine, nephrology.

INTRODUCTION

The spondyloarthritis (SpAs) comprise a set of rheumatic diseases of immune origin and familial pattern, having some characteristics in common, such as inflammatory axial pain, peripheral arthritis, enthesitis, and uveitis associated with sacroiliitis in genetically predisposed individuals (related to the human histocompatibility antigen HLA-B27). That set of diseases includes ankylosing spondylitis (AS), psoriatic arthritis (PsA), reactive arthritis (ReA), arthritis associated with inflammatory bowel disease (IBD), and undifferentiated spondyloarthritis (uSpA).
In that broad context, other organic systems can be involved. All conditions and symptoms not directly related to the articular system are considered extra-articular manifestations. Such manifestations can be divided into two groups as follows: those related to the concept of SpA, such as the involvement of the skin, eye, bowel or urogenital system; and those reflecting a long-term chronic inflammatory process, involving the lungs, heart, kidneys, and nervous system. The manifestations related to SpA are relatively frequent (20%–60%), can occur at any time of disease progression, and sometimes can be related to the axial and peripheral inflammatory process. On the other hand, the renal, cardiac, pulmonary and neurological manifestations are very rare (1%–5%), frequently subclinical, occurring usually in association with long-term disease and not related to the articular manifestations.

This study aimed at describing the prevalence of the extra-articular manifestations of SpA in Brazilian patients from a national databank, comprising the Brazilian’s major tertiary health care centers, focusing on the renal, pulmonary, cardiac and neurological manifestations.

MATERIALS AND METHODS

This is a cross-sectional descriptive study developed at several tertiary health care centers in Brazil participating in the Brazilian Registry of Spondyloarthritis (BRS), representing the five Brazilian geographic macroregions, with patients cared for from January 2006 to December 2009. A common protocol of investigation was applied to 1,472 consecutive patients diagnosed with SpA. The diagnosis of SpA was considered when the patients met the modified New York criteria. Psoriatic arthritis was considered when the patients met the Moll and Wright criteria. The diagnosis of ReA was considered when asymmetric inflammatory oligoarthritis of the lower limbs was associated with enthesopathy and/or inflammatory low back pain after enteric and/or urogenital infections. Arthritis associated with IBD was considered in the presence of inflammatory axial pain and/or peripheral articular involvement associated with IBD (Crohn disease or ulcerative colitis). Juvenile SpA was considered when the SpA symptoms began before the age of 16 years.

The patients were assessed for the following: prevalence of the major extra-articular manifestations (cardiac, pulmonary, renal, and neurological), which are shown in Table 1; clinical diagnoses (AS, PsA, ReA, arthritis associated with IBD, uSpA, and juvenile SpA); and the clinical forms (axial, peripheral, enthesitic, and mixed).

### Table 1

<table>
<thead>
<tr>
<th>Extra-articular manifestations</th>
<th>Clinical manifestations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiac</td>
<td>Aortitis, aortic insufficiency, conduction disorders; bundle-branch and atrioventricular blocks</td>
</tr>
<tr>
<td>Renal</td>
<td>Secondary amyloidosis; IgA nephropathy</td>
</tr>
<tr>
<td>Pulmonary</td>
<td>Fibrosis of the upper lobe and pleural thickening</td>
</tr>
<tr>
<td>Neurological</td>
<td>Cauda equina syndrome</td>
</tr>
</tbody>
</table>

RESULTS

Cardiac involvement

Of 1,472 patients diagnosed with SpA, 44 (3%) had cardiac involvement, mainly conduction disorders and bundle-branch blocks. Regarding the clinical diagnosis, cardiac involvement was more frequent in AS and PsA and no patient with arthritis associated with IBD was diagnosed with cardiac involvement (Table 2). Regarding the clinical forms, cardiac involvement was more frequently found in the mixed and axial forms (Table 3).

Pulmonary involvement

Pulmonary involvement was reported by 19 patients (1.3%), who had pulmonary fibrosis (apical and/or unspecific findings) and pleural thickening. Pulmonary changes clearly predominated in patients with AS (Table 2). Regarding the clinical forms, pulmonary involvement was more frequently found in the mixed and axial forms (Table 3).

Renal involvement

Renal involvement was identified in 17 patients (1.2%), some of whom were diagnosed with IgA nephropathy, while the others had chronic hematuria with no diagnostic elucidation. Among the patients with renal involvement, AS and PsA predominated (Table 2). Regarding the clinical forms, renal involvement was more frequently found in the mixed form (Table 3).

Neurological involvement

Thirteen patients (0.9%) had neurological involvement, mainly paresthesia of their lower limbs, which hindered deambulation. Five patients had the diagnosis of cauda equina syndrome confirmed. Among the patients with neurological involvement, AS (Table 2) and the mixed clinical form (Table 3) predominated.
Low prevalence of renal, cardiac, pulmonary, and neurological extra-articular clinical manifestations in spondyloarthritis: analysis of the BRS

### Table 2
Prevalence of the extra-articular manifestations in spondyloarthritis according to the clinical diagnosis

<table>
<thead>
<tr>
<th>Clinical diagnosis of spondyloarthritis (n = 1,472)</th>
<th>Extra-articular manifestations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cardiac (n = 44)</td>
</tr>
<tr>
<td>Primary AS</td>
<td>29 (2%)</td>
</tr>
<tr>
<td>AS + psoriasis</td>
<td>1 (0.1%)</td>
</tr>
<tr>
<td>PsA</td>
<td>11 (0.7%)</td>
</tr>
<tr>
<td>ReA</td>
<td>1 (0.1%)</td>
</tr>
<tr>
<td>uSpA</td>
<td>1 (0.1%)</td>
</tr>
<tr>
<td>AS + IBD</td>
<td>0</td>
</tr>
<tr>
<td>Arthritis + IBD</td>
<td>0</td>
</tr>
<tr>
<td>Juvenile SpA</td>
<td>1 (0.1%)</td>
</tr>
</tbody>
</table>

AS: ankylosing spondylitis; PsA: psoriatic arthritis; ReA: reactive arthritis; uSpA: undifferentiated spondyloarthritis; IBD: inflammatory bowel disease; Juvenile SpA: juvenile spondylarthritides.

### Table 3
Prevalence of the extra-articular manifestations of spondyloarthritis according to the clinical form

<table>
<thead>
<tr>
<th>Extra-articular manifestations</th>
<th>Clinical form of spondyloarthritis (n = 1,472)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cardiac (n = 44)</td>
</tr>
<tr>
<td>Cardiac (n = 44)</td>
<td>12 (0.8%)</td>
</tr>
<tr>
<td>Renal (n = 17)</td>
<td>2 (0.1%)</td>
</tr>
<tr>
<td>Pulmonary (n = 19)</td>
<td>9 (0.7%)</td>
</tr>
<tr>
<td>Neurological (n = 13)</td>
<td>2 (0.1%)</td>
</tr>
</tbody>
</table>

DISCUSSION

This is the first study describing the major extra-articular manifestations (cardiac, renal, neurological, and pulmonary) of SpA in a large number of Brazilian patients, representing all the five major geographic regions of the country. Systemic extra-articular manifestations, although rare, have high morbidity and, thus, should be diagnosed and treated early.

Cardiac symptoms in patients with AS are rarely reported; cardiac diseases occur in 2%–10% of those patients, mainly in those with long-term disease, and are not associated with joint disease activity. HLA-B27 positive individuals can have a syndrome characterized by aortic insufficiency (with isolated regurgitation and no stenosis) associated with aortic root dilation and fibrosis with retraction of the cusps, which could progress to complete atrioventricular block and endarteritis obliterans of the small arteries. Third-degree atrioventricular block requiring the use of pacemaker and with no defined cause in young men seems to be associated with a higher prevalence of HLA-B27,9 and can even be considered an uSpA.10 Currently, the occurrence of aortic insufficiency in SpA is not frequent,11,12 in accordance with the clinical findings of the present study. In this Brazilian cohort, cardiac involvement was observed in 3% of the 1,472 patients assessed, predominating in those with AS and PsA, with the mixed clinical form, followed by the axial form.

In recent years, both AS and PsA seem to be associated with an increase in cardiovascular morbidity and mortality.13,14 An extensive study assessing 28,208 patients with rheumatoid arthritis (RA), 3,066 patients with PsA, and 1,843 with AS, and comparing them with the healthy North-American population has revealed that both the cardiovascular diseases and their risk factors are more frequent in RA, PsA, and AS as compared with the paired control group.15 That Brazilian study has not assessed actively the occurrence of metabolic syndrome in patients with SpA, which can be studied in the second phase of the BRS.

Pulmonary involvement in AS is unusual and estimated to occur in less than 1% of the patients, especially in severe long-term disease.16 We observed 1.3% of pulmonary involvement in patients with SpA, with predominance of unspecified fibrotic changes in the high-resolution computed tomography of patients with AS. Tomographic studies, usually in patients with AS, have also reported unspecified tomographic changes.17-19 Usually such changes have no direct relationship with disease progression. Involvement of the costovertebral joints and ankylosis of the thoracic vertebral column will lead to limitation of the thoracic expansion, which can affect the patient’s respiratory capacity, but rarely leads to restrictive respiratory disorder, because the diaphragmatic function is preserved.

The symptomatic renal involvement is usually rare in patients with SpA. IgA nephropathy has been described in spondylitic patients for decades, being characterized by hematuria and proteinuria (usually mild), with mesangial IgA deposits on the renal biopsy, and no poor prognosis.20 The most frequent finding of the renal involvement in SpA is hematuria, usually microscopic; in such cases, in addition to IgA nephropathy, interstitial nephropathy due to non-steroidal anti-inflammatory drugs or renal lithiasis should be considered.21-23 In this Brazilian multicenter study, only 1.2% of the patients had renal involvement, mainly chronic hematuria with no specific cause. A recent Brazilian study has reported intermittent microscopic hematuria in 44.7% of 76 spondylitic patients, of whom only 10.5% could have a glomerular etiology.24

Renal amyloidosis, not described in this cohort and very rarely described in Latin-American case series,25 is usually more often reported in European patients with SpA. Renal


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amyloidosis was the cause of death in 13% of the spondylitic patients of a Finnish case series followed up for a long time. A Spanish study using the method of abdominal fat aspiration has found amyloidosis in 7% of the 107 patients assessed.

The neurological involvement in SpA is a clinical manifestation found almost exclusively in AS. In our cohort, of the 13 patients with neurological manifestations, eight had AS. The neurological changes more often reported in the literature are atlantoaxial subluxation and cauda equina syndrome. A recent literature review assessing the evolution of the neurological complications after vertebral column fracture has revealed a 17.7% mortality after three months.

In conclusion, extra-articular manifestations (cardiac, renal, pulmonary, and neurological) are extremely rare in Brazilian patients with SpA, ranging from 0.9%–3%, being, thus, in accordance with data in the literature. Such manifestations should be considered in patients with refractory long-term disease. Their diagnosis requires strong clinical suspicion, because the symptoms are unspecific and often subclinical. Initial check-up and regular control of such manifestations should, thus, be part of those patients’ follow-up.
espondilíticos, dos quais apenas 10,5% dos casos poderiam ter etiologia glomerular.24

A amiloidose renal, não descrita nesta coorte e muito raramente descrita nas séries latino-americanas,25 costuma ser mais frequente em pacientes europeus com EpA. Ela foi causa de óbito em 13% dos pacientes espondilíticos de uma coorte finlandesa acompanhada por longo prazo.26 Estudo espanhol utilizando o método de aspiração de gordura abdominal encontrou amiloidose em 7% de 107 pacientes avaliados.27

O envolvimento neurológico nas EpA é uma manifestação clínica encontrada quase exclusivamente na EA. Em nossa coorte, dos 13 pacientes que apresentaram manifestações neurológicas, oito tinham EA. As alterações neurológicas descritas na literatura com mais frequência são a subluxação atlantoaxial e a síndrome da cauda equina.28,29 Uma revisão recente da literatura avaliando a evolução das complicações neurológicas após fratura de coluna revelou mortalidade de 17,7% após três meses.30

Em conclusão, manifestações extra-articulares (cardíacas, renais, pulmonares e neurológicas) são extremamente raras nas EpA em pacientes brasileiros, variando de 0,9%–3%, condizente com os dados da literatura. Tais manifestações devem ser consideradas em pacientes com doença refratária e de longa evolução. O diagnóstico exige forte grau de suspeição clínica, em virtude de os sintomas serem inespecíficos e muitas vezes subclínicos; check-up inicial e controle regular dessas manifestações devem, portanto, ser parte necessária do acompanhamento desses pacientes.

REFERENCES
REFERÊNCIAS
Baixa prevalência das manifestações extra-articulares renais, cardíacas, pulmonares e neurológicas nas espondiloartites: análise do RBE

ABSTRACT

The authors reviewed the influence of nutritional factors on systemic lupus erythematosus (SLE) and discussed an alternative treatment option. The autoimmunity and inflammatory process of SLE are related to the presence of dyslipidemia, obesity, systemic arterial hypertension, and metabolic syndrome, which should be properly considered to decrease cardiovascular risk. A diet with moderate protein and energy content, but rich in vitamins, minerals (especially antioxidants), and mono/polyunsaturated fatty acids can promote a beneficial protective effect against tissue damage and suppression of inflammatory activity, in addition to helping the treatment of those comorbidities. Diet therapy is a promising approach and some recommendations may offer a better quality of life to patients with SLE.

Keywords: systemic lupus erythematosus, diet, nutrition assessment, nutrition programs.

INTRODUCTION

Systemic lupus erythematosus (SLE) is a multisystemic chronic inflammatory disease of unknown cause and autoimmune nature, characterized by the presence of several autoantibodies. In addition to the specific aspects related to its medicamentous treatment, some supportive measures, such as instructions about the disease, psychosocial support, physical activity, and especially the dietary approach, are essential to provide comprehensive health care to patients with SLE. In fact, diet can help to control the inflammatory findings of the disease and the complications derived from its own therapy. Considering that the cardiovascular risk seems increased in patients with SLE due to the increased frequency of conditions associated with atherosclerosis, such as dyslipidemia, diabetes mellitus (DM), metabolic syndrome (MS), and obesity, dietary guidance is important to minimize those complications of the disease.

The autoimmunity and the inflammatory process of SLE are directly related to changes in the lipid profile and to the metabolism of lipoproteins in SLE. The dyslipoproteinemia of the disease is characterized by higher levels of triglycerides (TG) and very-low-density lipoprotein cholesterol (VLDL-C) associated with lower levels of high-density lipoprotein cholesterol (HDL-C). Patients with both active and inactive disease show those lipid changes, which are aggravated by the higher inflammatory activity of the disease, demonstrating that SLE by itself promotes a proatherogenic lipoprotein profile. A reduction in the enzymatic activity of lipoprotein lipase is responsible for determining a dyslipoproteinemia characteristic of the disease, because it reduces the catabolism of TG-rich lipoproteins (chylomicrons and VLDL-C) due to either the presence of anti-lipoprotein lipase antibodies (anti-LPL) or the action of the tumor necrosis factor-α (TNF-α).

Several drugs used to treat SLE determine deleterious changes in the lipid profile previously altered by the disease itself, the effect of corticosteroids being of particular importance. Their chronic use in SLE is associated with an increase in total cholesterol and its fractions and TG, which can be
observed after 1–2 months of treatment.\textsuperscript{2} It is already known that, for each 10-mg/day increase in the dose of prednisone, a 7.5-mg\% elevation in total cholesterol is observed.\textsuperscript{7} In addition, corticosteroids induce the appearance of other risk factors, such as obesity, systemic arterial hypertension (SAH), hyperinsulinemia, and insulin resistance.\textsuperscript{2,8}

Hyperinsulinemia increases oxidative stress, which is considered an important pathophysiological mechanism for the development of atherosclerosis. Some studies have evidenced that DM is significantly more common in patients with SLE than in the general population, because of the reduced insulin sensitivity, and that approximately 18\%–38\% of the patients have MS.\textsuperscript{2,9,10}

It is worth noting that more than half of the patients with SLE have three or more risk factors for cardiovascular disease, particularly obesity, SAH, and dyslipidemias, suggesting that they are really more susceptible to the MS.\textsuperscript{2,11} A Brazilian assessment of the nutritional status of 170 patients with SLE has reported a 1.2\% prevalence of grade I thinness and a 64.2\% prevalence of excessive weight (35.9\% of overweight; 21.8\% of grade I obesity; 41.1\% of grade II obesity; 2.4\% of grade III obesity). Eutrophy, according to the Body Mass Index (BMI), has been observed in only 34.7\% of the patients assessed, leading to the conclusion that excessive weight is a frequent finding during the follow-up of patients with SLE.\textsuperscript{12} Thus, it is extremely important to establish strategies, such as programs to encourage the practice of physical activity and body weight reduction, in addition to nutritional counseling, to reduce the risks of MS.

In addition, the hyperlipid diet (rich in cholesterol and saturated fat) is one of the major factors for maintaining dyslipidemia in SLE, perpetuating and aggravating lipid profile changes.\textsuperscript{3,13,14} On the other hand, antioxidant nutrients, such as \(\beta\)-carotene, \(\alpha\)-tocopherol, ascorbic acid, and selenium are known to protect against tissue damages by both activating macrophages, monocytes and granulocytes, and suppressing the activity of cytokines and TNF-\(\alpha\).\textsuperscript{15}

Diet therapy is a promising way to approach SLE, with the indication of vitamin- and mineral-rich foods (mainly the antioxidant ones) and mono/polyunsaturated fatty acids, and moderate energy consumption, aiming at reducing inflammatory markers and helping in the treatment of those comorbidities and of the adverse reactions to drugs.\textsuperscript{11,13,16,17}

**DIET THERAPEUTIC INTERVENTIONS IN SYSTEMIC LUPUS ERYTHEMATOSUS**

The dietary status refers to the intake of nutrients from food and from supplements, being part of the nutritional status. The nutritional status is extremely important to the immune system balance, the diet composition assuming a fundamental role in maintaining the health of all individuals, including those with SLE.

In fact, thinness or low weight can indicate chronic energy deficiency, being, thus, associated with greater morbidity and mortality.\textsuperscript{12,18} Overweight and obesity can also be harmful to health, depending on their duration and severity, because these factors reduce resistance, favoring infections.\textsuperscript{12,18} Because of their deleterious effects on the immune function, both disorders should be diagnosed, aiming at improving the quality of life.

In addition, there is evidence that dietary factors can contribute to the geoepidemiology of autoimmune diseases.\textsuperscript{19} An adequate diet can, thus, be an essential factor to improve the prognosis of immune diseases, in addition to helping to prevent infections and the progression of cardiovascular diseases.

**Calories**

The restriction of calories in the diet alters the progression of autoimmune diseases.\textsuperscript{20} Some studies have shown that energy restriction around 30\%–40\% of the food intake prolongs the life of MRL/lpr mice by inhibiting the development of the lymphoproliferative syndrome, with a reduction in the secretion of IgG 2A (the major antibody of the autoimmune nephritis due to renal deposits) and of the platelet-derived growth factor (PDGF), which can reduce the glomerular lesion of NZB/NZW mice.\textsuperscript{17,21–23}

Energy restriction inhibits the decrease of CD4\(^+\) and CD8\(^+\) lymphocytes, in addition to attenuating the increase in Th1 cytokines (IL-2 and interferon-\(\gamma\) (IFN-\(\gamma\))) produced in NZB/NZW mice.\textsuperscript{24}

The National Academy of Sciences recommends the intake of 1,800–2,000 calories/day for a sedentary eutrophic adult, and of 2,200–2,500 calories/day in the presence of minimum physical activity.\textsuperscript{24} Regarding the treatment of excessive weight, the assessment of 86 studies performed by the US National Institutes of Health\textsuperscript{25} has shown that a diet of 1,000–1,200 kcal/day results in the loss of 7–13 kg (mean of 8\%) in 3–6 months, with a mean 10-cm reduction in abdominal fat in 6–24 weeks.\textsuperscript{25} That recommendation is also strongly supported by the British Nutrition Foundation.\textsuperscript{26} The National Cholesterol Education Program shares the same opinion, recommending a deficit of 500–1,000 kcal/day by use of a diet of 1,000–1,200 kcal/day for women and 1,200–1,400 kcal/day for men.\textsuperscript{27}

The excessive weight particularly observed in patients with SLE on chronic corticosteroids determines a higher probability of cardiovascular diseases, generating a vicious circle, in which weight gain can maintain disease activity, requiring the continuation of corticosteroids.\textsuperscript{22}
Table 1 shows the major favorable and unfavorable aspects regarding calories in the treatment of SLE. The major food sources indicated in Table 1 are found in the USDA National Nutrient Database for Standard Reference.28

Protein

Studies have shown that moderate-protein diet-fed mice had a long-lasting immune function and a delay in the autoimmunity development as compared with normal-protein diet-fed mice. A diet with restriction of the amino acids phenylalanine and tyrosine was beneficial to NZB/NZW mice.8,20

Supplementation with royal jelly (a honeybee secretion) has also been considered beneficial.29 Its composition rich in free amino acids, simple carbohydrates, proteins, short-chain fatty acids, and vitamins causes a reduction in cholesterol and has immunomodulating and anti-inflammatory activities. In fact, the royal jelly supplementation has induced a reduction in the IL-10 serum levels and has increased the life span of NZB/NZW mice, suppressing the disease symptoms.29

In human beings, the study by Caetano et al.8 has revealed that excessive protein intake causes a constant bone mineral loss in patients with juvenile SLE. On the other hand, the consumption of a protein-restricted diet (0.6 g/kg/day) has improved the glomerular filtration rate in the prediabetic chronic kidney disease of patients with systemic diseases.30 It is worth noting that, in lupus nephritis, a hypoprotein diet is not recommended to prevent negative nitrogen balance and malnutrition.31

Isoflavones

Because soybean-based foods have high levels of isoflavones, whose structure is similar to that of 17β-estradiol (E2), they have estrogenic effects and reduce proteinuria and the renal lesions associated with progressive renal failure.17,32 However, potent adverse effects of isoflavones on the immune response of mice have also been reported.33

Zhao et al.33 have reported that a soybean-rich diet can exacerbate renal damages, increasing serum creatinine and reducing the creatinine clearance, which increase the severity of the glomerular disease in MPL/lpr mice. The results have shown that soybean can accelerate glomerulonephritis, but improves the proliferative function of T cells.33

On the other hand, Hong et al.32 have shown that the supplementation with isoflavones increased the survival of SLE murine models, inhibiting the production of autoantibodies (anti-dsDNA and anticardiolipin), and reducing the secretion of IFN-γ. Those authors have also reported that isoflavones have anti-inflammatory properties and antioxidant effects.17,32

L-canavanine

This non-protein amino acid can be found in grains (soybean), onion, seeds and sprouts of alfalfa (major source) and other plants. It is a natural L-arginine homologue that acts with antimetabolic activity and whose presence can result in cell apoptosis in conditions of arginine deficiency.14 Studies have demonstrated that the L-canavanine amino acid acts as a suppressor-inductor of T cells that regulate the synthesis of antibodies and the proliferation of lymphocytes.20

Alfalfa sprouts have high levels of fibers and prevent hypercholesterolemia and atherosclerosis in some SLE models.21 Hong et al.17 have concluded that supplementation with ethylacetate extract of alfalfa in murine models for SLE reduced significantly the secretion of IFN-γ, reducing the inflammatory risk and immune mediators. However, some studies with human volunteers and healthy cynomolgus monkeys have shown that the intake of alfalfa sprouts can induce a lupus-like autoimmune syndrome (with antinuclear antibodies, anti-dsDNA, and complement reduction), and its discontinuation induces remission.17,34

The Baltimore Lupus Environmental Study (BALES) has also shown a significant association between alfalfa sprout consumption and the appearance of SLE, suggesting that none of its derivatives should be used.22,34 In addition, in patients with SLE and inactive disease, the consumption of 8–15 tablets of alfalfa per day has been shown to reactivate the clinical symptoms of the disease and its serological aspects.17

Although the findings have suggested that alfalfa derivatives should not be used in SLE, it is worth noting that cooking and autoclaving apparently destroy their deleterious effects without damaging their lipid-lowering properties.16

Taurine

Taurine is the major free intracellular β-amino acid found in mammal tissues that can be synthetized through methionine and cysteine, being found mainly in foods, such as eggs, meat, oyster, and squid. Taurine exerts an important protective function, because, in addition to regulating the immune response, reduces oxidative stress, inflammatory cytokines, and apoptosis, and reduces the serum levels of lipids and their oxidation in mice.35,36

Huang et al.35 have shown that taurine supplementation in NZB/NZW mice fed a hypercholesterolemic diet has reduced cardiac abnormalities, such as histopathologic changes, increased apoptosis, and fibrosis. Taurine has been commonly indicated for the treatment of myocardial failure, hepatic abnormalities...
Table 1
Favorable and unfavorable aspects of calories, proteins and amino acids in systemic lupus erythematosus

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Favorable</th>
<th>Unfavorable</th>
<th>Sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calories</td>
<td>Restriction&lt;br&gt;Inhibits the reduction of CD4&lt;sup&gt;+&lt;/sup&gt; and CD8&lt;sup&gt;+&lt;/sup&gt; T lymphocytes and attenuates ↑ of Th1 (IL-2 and IFN-γ)&lt;sup&gt;22&lt;/sup&gt; ↓ progression of autoimmune diseases&lt;sup&gt;24&lt;/sup&gt; ↓ secretion of IgG 2A&lt;sup&gt;17,21-23&lt;/sup&gt;</td>
<td>Excessive consumption&lt;br&gt;Metabolic syndrome&lt;br&gt;Higher risk of cardiovascular diseases&lt;br&gt;Disease activity&lt;sup&gt;25&lt;/sup&gt; ↑ weight and obesity</td>
<td>Foods and/or preparations rich in simple carbohydrates and fat</td>
</tr>
<tr>
<td>Protein</td>
<td>Moderate consumption&lt;br&gt;Better immune function&lt;br&gt;Delay in autoimmunity&lt;sup&gt;6,20&lt;/sup&gt;</td>
<td>Excessive consumption&lt;br&gt;Creatinine clearance in chronic renal failure in SLE (content &gt; 0.6 g/kg/day)&lt;sup&gt;10&lt;/sup&gt; Restriction&lt;br&gt;Negative nitrogen balance in lupus nephritis&lt;br&gt;Malnutrition&lt;sup&gt;13&lt;/sup&gt;</td>
<td>Meat**, dairy products**, eggs**, pulses, whole cereals*</td>
</tr>
<tr>
<td>Supplementation with royal jelly</td>
<td>Immunomodulatory and anti-inflammatory effect&lt;br&gt;↓ serum levels of IL-10&lt;br&gt;↓ SLE symptoms (experimental)&lt;sup&gt;26&lt;/sup&gt;</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Isoflavones</td>
<td>Anti-inflammatory and antioxidant effects&lt;sup&gt;17,22&lt;/sup&gt; ↓ autoantibody production &lt;sup&gt;(anti-dsDNA)&lt;sup&gt;17,26&lt;/sup&gt;&lt;/sup&gt; ↓ IFN-γ secretion ↓ proteinuria</td>
<td>↑ creatinine&lt;sup&gt;11&lt;/sup&gt;</td>
<td>Soybeans and derivatives**, dietary supplements, morning cereals*, black beans, olive oil*</td>
</tr>
<tr>
<td>L-canavanine</td>
<td>Prevents hypercholesterolemia&lt;sup&gt;(experimental)&lt;sup&gt;17,24&lt;/sup&gt;&lt;/sup&gt; ↓ cell apoptosis&lt;sup&gt;14&lt;/sup&gt;</td>
<td>Lupus-like in humans&lt;br&gt;Serologic reactivation&lt;sup&gt;17,22,24&lt;/sup&gt;</td>
<td>Alfalfa**, seeds*, onion*, soybeans*</td>
</tr>
<tr>
<td>Taurine</td>
<td>Protective effect against free radicals&lt;sup&gt;36&lt;/sup&gt; ↓ oxidative stress ↓ inflammatory cytokines and apoptosis&lt;sup&gt;15&lt;/sup&gt; ↓ lipids and lipid oxidation&lt;sup&gt;(experimental)&lt;sup&gt;15,27&lt;/sup&gt;&lt;/sup&gt;</td>
<td>NA</td>
<td>Eggs**, meat**, oyster**</td>
</tr>
</tbody>
</table>

NA: information not available. *Sources with lower contents; **Major sources.

Associated with SLE, and liver damages of patients with chronic hepatitis, when used at the dosage of 10 g/kg of weight in the diet of animals and 1 g/kg of weight for human beings.<sup>15,36</sup>

Several studies have shown the protective effect of taurine against free-radical damages, in addition to the inhibition of the hepatic apoptosis induced by biliary acids in mice. However, the mechanism of those effects have not been clearly established.<sup>36</sup>

Table 1 shows the major favorable and unfavorable aspects regarding proteins and amino acids in the treatment of SLE, and their major food sources according to the USDA National Nutrient Database for Standard Reference.<sup>28</sup>

**Lipids**

Lipids are important because they provide polyunsaturated fat to the tissues so that lymphocytes can exert their functions properly. Restriction of saturated fat and increase in the intake of unsaturated fat are recommended, because of the important role of unsaturated fat in the immune system and its response to cancer and infectious diseases.<sup>18</sup>

Dietary lipids influence the concentration and composition of plasma lipoproteins; saturated fats and omega-6 polyunsaturated fatty acid (ω-6 PUFA) can drastically affect autoimmune diseases in mice.<sup>18,20</sup> The total, saturated and monounsaturated fats are not associated with the appearance of DM. The higher intake of polyunsaturated fats reduces the risk of DM, while that of trans fats increases that risk – however, its minimal consumption can reduce that risk in as much as 40%.<sup>37</sup> Halen et al. have shown that a hyperlipid diet induces atherosclerosis in MRL/lpr and MRL/n mice.

On the contrary, food lipid restriction reduces the expression of immune complexes in glomerulonephritis and prolongs the life span of NZB/NZW mice.<sup>21</sup> In addition, dietary lipids can change the balance between Th1 and Th2 cells, favoring the development of autoimmune phenomena.<sup>18,21</sup>

Rev Bras Reumatol 2012;52(3):384-408
ω-3 and ω-6 polyunsaturated fatty acids

The eicosapentaenoic (EPA) and docosahexaenoic (DHA) unsaturated fatty acids inhibit the enzyme lipoygenase, reducing the production of inflammatory eicosanoids derived from the arachidonic acid. The DHA has a significant inhibitory action on the nuclear factor κB (NF-κB) and TNF-α, being even more potent than EPA. In addition, DHA significantly reduces the serum levels of anti-dsDNA, regulates IgG renal deposits in NZB/NZW mice, and reduces IL-1β. Halade GV et al. have reported a significant increase in the life span of NZB/NZW females by reducing the transforming growth factor β (TGF-β), renal mRNA, and protein. EPA is considered a potent anti-inflammatory agent because it reduces the production of interleukins (IL-1β and IL-6) and of TNF-α, by changing the phospholipid composition of the cell membrane, inhibiting the production and the receptor interaction of inflammatory cytokines. It is worth noting that a daily dose of 6 g of ω-3 PUFA for 10 weeks can cause a decrease of 4.6 mmHg in systolic blood pressure and of 3.0 mmHg in diastolic blood pressure in patients with SAH.

Flaxseed oil, with 70% of ω-3 PUFA in its composition and rich in ALA, is a good dietary complement, because it reduces proteinuria levels and preserves glomerular filtration, in addition to reducing anti-dsDNA and anticardiolipin antibodies in mice and suppressing the anti-β2-glycoprotein I in the experimental model of the antiphospholipid syndrome. That effect has not been found with the supplementation of other oils, such as those from safflower, Juniperus virginiana, fish, corn, and soybean, suggesting that the flaxseed oil has another protective component not completely identified, besides ω-3 PUFA. Flaxseed can also inhibit the platelet activating factor, commonly elevated in the inflammatory response of patients with SLE. It should be consumed in its whole form. The daily dosage of 30 g proved to be beneficial in reducing serum creatinine in patients with lupus nephritis, in addition to promoting a reduction of 11% in total cholesterol and LDL-C, in addition to reducing the severity of both autoimmunity and nephritis in NZB/NZW mice. Murine models of SLE have shown that the reduction in the consumption of ω-9 monounsaturated fatty acid and the increase in ω-3 PUFA have potentiated the therapeutic effect.

Supplementation with primrose oil can increase the life span of MRL/1pr, NZB/NZW, and BXSB mice, mainly because of its content of GLA (19%), from which prostaglandin E₂ (PGE₂), which has an anti-inflammatory action and reduces lymphocytic activity, is formed. Fish oil, known as one of the major sources of ω-3 PUFA, has anti-inflammatory and anti-autoimmune (due to inhibition of T and B lymphocytes) effects. In addition, it suppresses the activity of macrophages and the production of cyclooxygenase metabolites, being significantly beneficial to the clinical, immune, and biochemical status in animal and human models of SLE. Supplementation with fish oil as the exclusive source of lipids reduces proteinuria and protects the kidneys against the deleterious effects of free radicals in NZB/NZW, BXSB, and MRL/1pr mice with lupus nephritis, by inhibiting PI3K lipid kinase (an important target for reducing glomerulonephritis). In addition to reducing anti-dsDNA levels, its major benefit in SLE is due to its effect on apoptosis. Chandrasekar et al. have shown that supplementation with fish oil improves glomerulonephritis in NZB/NZW females by reducing the transforming growth factor β (TGF-β), renal mRNA, and protein.

Table 2 shows the major favorable effects regarding specific foods, sources of ω-3 PUFA, in the treatment of SLE. In healthy human beings, dietary supplementation with ω-3 PUFA causes a reduction in the production of IL-1α, IL-1β, IL-2, IL-6, NF-κB, and TNF-α. Several clinical studies have also shown that the consumption of ω-3 PUFA delays renal disease progression by reducing inflammation. A study with 12 patients with lupus nephritis and supplemented with...
Diet and nutritional aspects in systemic lupus erythematosus

Fish oil (180 mg of EPA and 120 mg of DHA) has reported a reduction in the following: arachidonic acid; inflammatory status; platelet aggregation; blood viscosity; and leukotriene B₄.²² It is worth noting that high doses (18 g/day) of fish oil reduce TG by 38% and increase HDL-C by 28%.¹⁶,²²

Patients with SLE have a reduced concentration of GLA, ALA, EPA, and DHA in the phospholipid fraction, in addition to reduced levels of nitric oxide, which increase when those patients are supplemented with EPA/DHA.⁴² Mohan et al.³² and Pestka et al.⁴⁹ have reported a significant increase in the levels of the antioxidant enzymes superoxide dismutase and glutathione peroxidase and an increase in the hepatic catalase levels of NZB/NZW mice with the EPA/DHA supplementation, inducing SLE remission and being beneficial in the treatment of lupus nephritis with cyclophosphamide.⁴⁹,⁴⁶ In vitro studies have revealed that the supplementation with GLA or arachidonic acid inhibits the production of IL-2. On the other hand, EPA has shown less inhibition of IL-2, indicating the immunosuppressive role of the ω-3 PUFA.⁴³

Table 3 shows the major favorable and unfavorable aspects regarding lipids and fatty acids in the treatment of SLE, and their major food sources according to the USDA National Nutrient Database for Standard Reference.²⁸

Vitamins

Vitamin A

The metabolites of vitamin A, such as retinoic acid, have an antineoplastic and regulatory role in cell proliferation and differentiation, in addition to increasing T cell cytotoxicity and proliferation and manifesting significant defects in Th cell activity.¹⁹,⁴⁸ They also have therapeutic effects on several animal models of renal diseases, such as lupus nephritis.⁴⁸

The study by Ikeda et al.⁴⁹ with MRL mice has shown that vitamin A derivatives, such as etretinate (synthetic retinoic acid) and retinoids, have significantly reduced dermal thickening and proved to be therapeutic agents in cutaneous T cell lymphoma and cutaneous basal cell carcinoma.

Fish oil (180 mg of EPA and 120 mg of DHA) has reported a reduction in the following: arachidonic acid; inflammatory status; platelet aggregation; blood viscosity; and leukotriene B₄.²² It is worth noting that high doses (18 g/day) of fish oil reduce TG by 38% and increase HDL-C by 28%.¹⁶,²²

Patients with SLE have a reduced concentration of GLA, ALA, EPA, and DHA in the phospholipid fraction, in addition to reduced levels of nitric oxide, which increase when those patients are supplemented with EPA/DHA.⁴² Mohan et al.³² and Pestka et al.⁴⁹ have reported a significant increase in the levels of the antioxidant enzymes superoxide dismutase and glutathione peroxidase and an increase in the hepatic catalase levels of NZB/NZW mice with the EPA/DHA supplementation, inducing SLE remission and being beneficial in the treatment of lupus nephritis with cyclophosphamide.⁴⁹,⁴⁶ In vitro studies have revealed that the supplementation with GLA or arachidonic acid inhibits the production of IL-2. On the other hand, EPA has shown less inhibition of IL-2, indicating the immunosuppressive role of the ω-3 PUFA.⁴³

Table 3 shows the major favorable and unfavorable aspects regarding lipids and fatty acids in the treatment of SLE, and their major food sources according to the USDA National Nutrient Database for Standard Reference.²⁸

Vitamins

Vitamin A

The metabolites of vitamin A, such as retinoic acid, have an antineoplastic and regulatory role in cell proliferation and differentiation, in addition to increasing T cell cytotoxicity and proliferation and manifesting significant defects in Th cell activity.¹⁹,⁴⁸ They also have therapeutic effects on several animal models of renal diseases, such as lupus nephritis.⁴⁸

The study by Ikeda et al.⁴⁹ with MRL mice has shown that vitamin A derivatives, such as etretinate (synthetic retinoic acid) and retinoids, have significantly reduced dermal thickening and proved to be therapeutic agents in cutaneous T cell lymphoma and cutaneous basal cell carcinoma.
because of their apoptosis-inducing action. Those mice, treated with 5 mg/kg and 10 mg/kg of etretinate, have not even had the characteristic cutaneous and dermatological lupus-like lesions, probably due to its suppressive effect on cytokine expression.\(^{49}\)

Other recent studies have shown that retinoids inhibit the formation of proinflammatory Th17 cells and promote the production of anti-inflammatory regulatory T cells in murine models of autoimmune diseases.\(^{15}\) Kinoshitak et al.\(^{48}\) have shown that patients treated with retinoids improved their proteinuria, their high levels of anti-dsDNA, and low titers of complements, with no side effects, suggesting that retinoids can be promising for the treatment of lupus nephritis.

The supplementation of vitamins A and D in patients with SLE can be indicated. The dose of 100,000 IU of vitamin A for two weeks has proved to be beneficial for the increased antibody-dependent cell cytotoxicity, activity of natural killer cells, and IL-2 response.\(^{16}\) However, the consumption of extremely high doses of vitamin A (≥100,000 IU) can result in symptoms such as anemia, headache, dry skin, alopecia, nausea, anorexia, pseudo hydrocephalus, and death. On the other hand, the deficiency of vitamin A in experimental models of SLE has shown greater severity of symptoms. The researchers have attributed that fact to the increase in hypergammaglobulinemia and presence of autoantibodies.\(^{20}\)

### Vitamin D

Vitamin D, an important nutrient due to its multiple immunomodulating effects, is produced in the skin and obtained from food. The effects of its active form \([1,25(OH)2D3]\) on immune response occur due to the inhibition of the proliferation of T lymphocytes (Th1).\(^{47,50}\) The treatment of CD4 T cells with \(1,25(OH)D_3\) inhibits the proliferation of Th1 cells and the production of cytokines, reduces the secretion of IL-2 and IFN-γ by the CD4 T cells, and promotes the production of IL-5 and IL-10, determining a shift toward a Th-2 response.\(^{50}\)

The high consumption of vitamin D (≥37 ng/mL) has been associated with the reduction in the risks for type I DM, autoimmune encephalomyelitis, SAH, hypertriglyceridemia, MS, inflammatory bowel disease, SLE, and multiple sclerosis.\(^{51–53}\) A recent prospective study carried out with 18,000 women during 22 years has found no association between vitamin D intake and risk for SLE, disagreeing with the hypothesis that the high vitamin D intake would be associated with protection against SLE.\(^{51}\) However, other epidemiological evidence supports the association between vitamin D and the severity of those autoimmune diseases.\(^{53}\)

Patients with SLE have been shown to have several factors that reduce vitamin D levels (≤20 ng/mL), which does not occur with patients with rheumatoid arthritis (RA) and osteoarthritis.\(^{16,50,51,53,54}\) In addition to the relative hypoparathyroidism caused by the high IL-6 levels (mainly in disease activity) and the chronic use of steroids, which changes its metabolism leading to the formation of biologically inactive metabolites and decreasing calcium absorption.\(^{50,55,56}\) Some studies have also suggested that excessive weight is an important risk factor for vitamin D deficiency in SLE.\(^{50,55,56}\)

In addition to those factors, hydroxychloroquine seems to reduce the conversion of vitamin D\(_3\) into D\(_\alpha\), its biologically more active form. Antibodies antivitamin D have also been described in patients with SLE, being associated with anti-dsDNA antibodies, present during disease activity.\(^{50,53}\) It is worth noting that low levels of 25(OH)D are related to the highest scores of inflammatory activity in SLE (SLEDAI);\(^{50}\) on the other hand, its high levels (>36.8 ng/mL) are associated with greater bone mineral density (both in young and elderly individuals of both genders), according to data of the Third National Health and Nutrition Examination Survey.\(^{57}\)

Recent reviews have confirmed that patients with SLE have significantly low serum levels of 25(OH)D (close to 25.5 ± 12.1 nmol/L), while the minimum serum concentration recommended is 50–80 nmol/L.\(^{50,52,55,57}\) Supplementation of that vitamin is appropriate, because its better indicator \([1,25(OH)D_3]\) also plays a role in calcium homeostasis and immune regulation.\(^{16,54,55,57}\) Supplementation with vitamin D\(_3\) in MRL/lpr mice has yielded longevity, a reduction in proteinuria, improvement in bone health and a positive impact on immunity.\(^{50,52,58}\)

### Vitamin E

The combination of fish oil and vitamin E has an impact on several SLE mediators. Mice fed with fish oil and 75 IU of vitamin E showed a reduction in inflammatory cytokines, PGE\(_\alpha\), leukotriene B\(_\alpha\), and thromboxane B\(_\alpha\), to which a reduction in the following factors was added with the increase in vitamin E offer to 500 IU: IL-6; IL-10; IL-12; and TNF-α.\(^{16,47}\) The significant effects on IL-2, IL-4, and TNF-α, obtained through the supplementation with vitamin E and ω-3 PUFA, have suggested that oncogenic levels can be delayed.\(^{41}\)
Some studies with MRL/lpr mice have shown that treatment with vitamin E supplementation modulates the levels of inflammatory cytokines, delays the appearance of autoimmunity, and increases survival, but the treatment in patients with SLE is still controversial.\(^{20}\) Other studies have found effects of vitamin E supplementation on neither endothelial dysfunction nor lipid peroxidation.\(^{59}\)

Another factor to be considered regarding not only vitamin E, but also vitamin A and β-carotene, supplementation relates to the reduction in the levels of the antioxidants α-tocopherol, β-carotene and retinol found in patients with SLE and RA, suggesting important damage to the inflammatory process caused by free radicals.\(^{15,16}\) The adequate consumption of vitamins A and E is inversely related to the SLE activity, according to Minami et al.\(^{45}\) However, the recent study by Costenbader et al.\(^{15}\) has not supported the existence of a relationship between consumption of food antioxidants or supplements and the risk for developing RA or SLE in women.

**Vitamin B complex**

The study by Varghese et al.\(^{60}\) in mice has shown that immune therapy with folate minimizes the symptoms of SLE and prolongs life span.

The higher plasma levels of homocysteine might be associated with atherosclerosis in SLE, which reinforces the need for a higher consumption of vitamins B6 and B12 (in addition to folate), which are important cofactors in its metabolism and promote a reduction in homocysteine levels.\(^{51}\) In addition, those vitamins also influence the serum levels of some inflammatory markers, such as cytokines and C-reactive protein (CRP).\(^{61}\)

Some studies have shown that the consumption of vitamin B12- and folate-deficient diets has caused a plasma increase in homocysteine in patients with SLE. Thus, it has been suggested that patients on a hypolipid diet (indicated for SLE) should increase their consumption of cereals fortified with those nutrients, in addition to vegetables and fruits. The possibility of supplementation should also be considered.\(^{62}\)

The study by Minami et al.\(^{61}\) has shown the association of high doses of vitamin B6 and folate with a lower severity of SLE in Japanese patients, regardless of the non-dietary factors. The study by Ardoin et al.\(^{53}\) has shown that niacin reduces TG and LDL-C levels by 23% and 30%, respectively, in children with dyslipidemia, although it does not significantly affect HDL-C levels.

**Vitamin C**

Studies with mice have suggested that vitamin C reduces IgG and anti-dsDNA levels and that its insufficient consumption can maintain oxidative stress and induce inflammation in the active phase of disease.\(^{45}\)

According to the study by Minami et al.\(^{45}\) carried out with 279 patients with SLE, vitamin C consumption is inversely associated with the risk of SLE inflammatory activity. The antioxidant properties of that vitamin modulate the immune functions and release of inflammatory mediators.\(^{42}\) Tam et al.\(^{59}\) have shown that the supplementation with antioxidants is a potent therapy to prevent cardiovascular complications. In fact, monthly vitamin C supplementation has determined a significant improvement in the mediation flow of vasodilation in patients with coronary artery disease.\(^{59}\) The safe maximum dose is 1 g/day, because the consumption of higher doses determines the appearance of ascorbate in urine. The combination of daily supplementation of vitamin C (500 mg) with vitamin E (800 IU) for three months was associated with a small reduction in lipid peroxidation, without affecting other markers of oxidative stress or endothelial function in patients with SLE.\(^{59}\)

Table 4 shows the major favorable and unfavorable aspects regarding vitamins for the treatment of SLE, and their major food sources according to the USDA National Nutrient Database for Standard Reference.\(^{28}\)

**Fibers**

Adequate intake of dietary fibers is recommended, because it lowers post-prandial glycemia and lipids, yielding low-energy-density nutrients. In addition, dietary fibers improve intestinal constipation, promoting satiety due to the longer chewing time.

Epidemiological studies have reported that fibers protect against cardiovascular diseases.\(^{64,65}\) In fact, 10 cohort studies in the United States and Europe, with a six-to-ten-year follow-up, have concluded that fibers were associated with a risk reduction of 14% and 27% in coronary events and coronary death, respectively.\(^{65}\) Those results can be explained by the effect of fibers on blood pressure and on CRP levels. The intake of fibers has been inversely associated with CRP in the National Health and Nutrition Examination Survey 1999–2000.\(^{66}\)

As foods are digested and absorbed in the small intestine, the fibers increase the viscosity in the intestinal lumen, interfering with the biliary acid absorption from the ileum. The LDL-C is removed from the blood and converted to biliary
acids to replace those eliminated with defecation. This change in the pool of biliary acids, along with the intake of viscous fibers, depress cholesterol synthesis. Simultaneously, inulin, oligosaccharides, resistant starch, and other fibers increase mineral absorption, especially that of calcium. The diet therapeutic intervention to control hypercholesterolemia and MS in SLE should emphasize the importance of consuming fiber-rich foods, especially the soluble ones (found in oat, fruits and pulses) to control dyslipidemia. The recommendation is 14 g of fibers per 1,000 kcal consumed (or 38 g for men and 25 g for women), adequate fluid ingestion being required.

Minami et al. have also reported that fiber intake was inversely proportional to the SLE severity risk. Some studies have already shown that fiber intake is inversely associated with the plasma levels of homocysteine and of the inflammatory markers IL-6 and CRP. However, excessive fiber intake reduces the absorption of vitamins, minerals, protein and energy.

Table 4 shows major favorable and unfavorable aspects regarding dietary fibers for the treatment of SLE, and their major food sources according to the USDA National Nutrient Database for Standard Reference.

Minerals

Zinc

MRL/lpr mice on a zinc-restricted diet have shown a reduction in lymphoproliferation and in anti-dsDNA titers, and an improvement in glomerulonephritis, as well as a reduction in the production of autoantibodies in NZB/NZW models. A zinc-restricted diet determines an increase in the serum levels of corticosteroids, which can contribute to control SLE. On the other hand, a study in human beings has shown that zinc deficiency causes an immune dysfunction that affects mainly Th cells, and can cause neurosensorial disorders and body mass reduction.

Selenium

A diet rich in selenium, a natural antioxidant, increases anti-inflammatory properties, with a reduction in anti-dsDNA antibodies, improving the activity of natural killer cells and survival in murine models of SLE. It can have a significant effect on the maturation of T cells and on the response of T cell-dependent autoantibodies.

Calcium

An adequate consumption of calcium is extremely important in SLE, particularly in patients with a reduction in bone mineral density either associated or not with corticotherapy and regardless of disease duration. The risk of osteoporosis is greater due to disease activity, vitamin D deficiency, non-exposure to sunlight, and early menopause caused by cytotoxic agents. In fact, women with SLE are five times more likely to undergo fractures as compared with healthy women of the same age.

The American College of Rheumatology (ACR) has issued recommendations to reduce bone mass loss in patients with SLE treated with corticosteroids. In addition, ACR has suggested that patients receiving more than 5 mg of prednisone daily, for three months, should begin to receive calcium and vitamin D prophylactically, and undergo assessment of bone density and of the use of other medications. Changes in life style and a calcium-rich diet have also been suggested. Supplementation of calcium (> 1,500 mg) and vitamin D (20 μg or 800 UI) is indicated in cases of difficulty in obtaining those nutrients from the diet.

Iron

Some studies have suggested that iron can cause cell damages and that the use of chelating agents has shown benefits in experimental models for autoimmune diseases. More severe renal lesions were more prevalent in mice being supplemented with iron as compared with controls; however, animals with mineral deficiency developed more severe clinical signs of the disease. That study has suggested that a dietary restriction would reduce mortality in those models.

On the other hand, some studies with patients with SLE have shown that anemia can be detected in up to 70% of them during the course of disease. The most often found type of anemia is that of chronic disease (characterized by deficient mobilization of iron to the bone marrow, despite the normal or increased values of iron reserves). In the case of iron-deficiency anemia, the consumption of the following is indicated: lean meat (mainly white); dark-green leafy vegetables; whole cereals; iron-fortified foods; and, in more severe cases, medicamentous supplementation.

Sodium

Patients with lupus nephritis and those with SAH, either secondary or not to corticotherapy, need to follow a sodium-restricted diet. The daily amount considered adequate and safe for those conditions is of 3 g sodium/day, and should be also followed by a restriction of fluid intake (maximum of 1.5 L/day).

Copper

High serum concentrations of copper have been observed in patients with SLE and RA, and such levels are directly related.
Table 4
Favorable and unfavorable aspects of vitamins, fibers and minerals in systemic lupus erythematosus

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Favorable</th>
<th>Unfavorable</th>
<th>Sources</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Vitamin A</strong></td>
<td>Retinoids</td>
<td>Antineoplastic agent&lt;sup&gt;48&lt;/sup&gt; &lt;br&gt;Induces apoptosis&lt;sup&gt;48&lt;/sup&gt; &lt;br&gt;↓ proteinuria and improves hypoalbuminemia&lt;sup&gt;48&lt;/sup&gt;</td>
<td>Very high doses</td>
</tr>
<tr>
<td></td>
<td>Supplement</td>
<td>↑ natural killer cell activity and response to IL-2&lt;sup&gt;16&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td><strong>Vitamin D</strong></td>
<td>Immunomodulatory effects&lt;sup&gt;16,55&lt;/sup&gt;</td>
<td>Inhibition of Th1 proliferation</td>
<td>Reduction in 25(OH)D&lt;sub&gt;3&lt;/sub&gt;</td>
</tr>
<tr>
<td></td>
<td>Calcium homeostasis&lt;sup&gt;16,55,57&lt;/sup&gt;</td>
<td>↓ IL-2, IFN-γ&lt;sup&gt;28&lt;/sup&gt; and ↓ proteinuria&lt;sup&gt;28,52,58&lt;/sup&gt;</td>
<td>Associated with high SLEDAI scores&lt;sup&gt;53&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>↑ IL-5 and IL-10&lt;sup&gt;50&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>High intake</td>
<td>↓ risk of type 1 DM, SAH, SLE&lt;sup&gt;61,62&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td><strong>Vitamin E</strong></td>
<td>Adequate intake</td>
<td>Inversely related to SLE activity&lt;sup&gt;45&lt;/sup&gt;</td>
<td></td>
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<tr>
<td></td>
<td>Supplementation</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Vitamin B complex</strong></td>
<td>B6 and B12</td>
<td>↓ homocysteine&lt;sup&gt;61&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Folate</td>
<td>Minimizes SLE symptoms&lt;sup&gt;61,62&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Folic acid</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Niacin</td>
<td>↓ triglycerides and LDL-C&lt;sup&gt;61&lt;/sup&gt;</td>
<td></td>
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<tr>
<td><strong>Vitamin C</strong></td>
<td>Antioxidant</td>
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<tr>
<td></td>
<td>Immune function modulator</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Release of inflammatory mediators&lt;sup&gt;41&lt;/sup&gt;</td>
<td></td>
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<tr>
<td></td>
<td>IgG and anti-diDNA&lt;sup&gt;50&lt;/sup&gt;</td>
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<tr>
<td><strong>Fibers</strong></td>
<td>Low-energy-density</td>
<td>Protection against cardiovascular diseases&lt;sup&gt;64&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>Protection against hypercholesterolemia&lt;sup&gt;62&lt;/sup&gt;</td>
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<td></td>
<td>Lower chance of metabolic syndrome&lt;sup&gt;62&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>↓ post-prandial glycemia and lipids</td>
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<td></td>
<td>↓ blood pressure and CRP&lt;sup&gt;64,66&lt;/sup&gt;</td>
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<td></td>
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<tr>
<td></td>
<td>↓ homocysteine and IL-6&lt;sup&gt;64&lt;/sup&gt;</td>
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<tr>
<td><strong>Zinc</strong></td>
<td>Restriction</td>
<td>↓ anti-diDNA titers</td>
<td></td>
</tr>
<tr>
<td></td>
<td>↓ autoantibodies (models)&lt;sup&gt;50&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>↓ symptoms of autoimmune diseases&lt;sup&gt;50&lt;/sup&gt;</td>
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<tr>
<td><strong>Selenium</strong></td>
<td>Antioxidant</td>
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<tr>
<td></td>
<td>Anti-inflammatory</td>
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<tr>
<td></td>
<td>Improves the activity of natural killer cells</td>
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<td></td>
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<tr>
<td></td>
<td>Longer survival in SLE (experimental)&lt;sup&gt;16,69&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Important in T cell maturation&lt;sup&gt;19&lt;/sup&gt;</td>
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<tr>
<td><strong>Calcium</strong></td>
<td>Important in bone mass reduction&lt;sup&gt;14,15,48&lt;/sup&gt;</td>
<td></td>
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<tr>
<td><strong>Iron</strong></td>
<td>Anemia prevention&lt;sup&gt;70&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Supplementation</td>
<td>Worsens renal damages</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Worsens SLE clinical findings&lt;sup&gt;60&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Sodium</strong></td>
<td>NA</td>
<td>SAH</td>
<td></td>
</tr>
<tr>
<td><strong>Copper</strong></td>
<td>Possible therapeutic effect on inflammatory diseases&lt;sup&gt;65&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NA: information not available. *Sources with lower contents; **Major sources; †Consumption not recommended.
to disease activity and probable inflammatory response. Copper is believed to exert a therapeutic effect on the treatment of chronic diseases because its liver storage is insufficient to meet the demands of the inflammatory response. Exogenous copper reduces cell formation in mice, but its supplementation has not produced significant serum changes in the study by Duffy et al.46

Table 4 shows major favorable and unfavorable aspects regarding minerals for the treatment of SLE, and their major food sources according to the USDA National Nutrient Database for Standard Reference.28

Table 4

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Favorable</th>
<th>Unfavorable</th>
<th>Recommended</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyunsaturated fatty acid (ω-3)</td>
<td>++</td>
<td>-</td>
<td>Oils of flaxseed and canola, fish oil, olive oil, salmon and sardine</td>
</tr>
<tr>
<td>Fibers</td>
<td>++</td>
<td>-</td>
<td>Whole cereals, pulses</td>
</tr>
<tr>
<td>Isoflavones</td>
<td>++</td>
<td>?</td>
<td>Soybeans and derivatives</td>
</tr>
<tr>
<td>Vitamin D</td>
<td>++</td>
<td>?</td>
<td>Vitamin D-fortified foods</td>
</tr>
<tr>
<td>Taurine</td>
<td>+</td>
<td>-</td>
<td>Eggs, meat</td>
</tr>
<tr>
<td>Vitamin A</td>
<td>+</td>
<td>-</td>
<td>Carrot, pumpkin</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>+</td>
<td>-</td>
<td>Whole cereals, nuts, fish</td>
</tr>
<tr>
<td>Vitamin B complex</td>
<td>+</td>
<td>-</td>
<td>Red meat, fortified cereals</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>+</td>
<td>-</td>
<td>Orange juice, tangerine, broccoli, papaya</td>
</tr>
<tr>
<td>Selenium</td>
<td>+</td>
<td>-</td>
<td>Nuts, whole cereals</td>
</tr>
<tr>
<td>Calcium</td>
<td>+</td>
<td>-</td>
<td>Dairy products, kale, sardine, spinach</td>
</tr>
</tbody>
</table>

+ Evidence; ++ Strong evidence; - Negative evidence; ? No data.
REFERENCES


67. Abrams SA, Grif...


Static and dynamic balance in subjects with ankylosing spondylitis: literature review

José Eduardo Pompeu1, Renata Sorroche Lourenço Romano2, Sandra Maria Alvarenga Anti Pompeu3, Sônia Maria Anti Loduca Lima4

ABSTRACT

To analyze the musculoskeletal changes of individuals with ankylosing spondylitis (AS) and their repercussions on postural control, a literature review was carried out in the BIREME and EBSCO HOTS databases and Pubmed site with the following keywords: “ankylosing spondylitis”, “postural balance”, and “posture”. Articles involving human beings, assessing the postural control and balance of individuals with AS, written in English or Portuguese and published between 1999 and 2010, were selected. Of the total number of articles found, only four met the requirements. Of those, three compared the outcomes of patients with AS with data obtained from healthy individuals, and one article assessed individuals with AS. No article used the same method of postural analysis. To assess balance, Berg Balance Scale, Force Plate, and Magnometry were used. The major postural deviations found were increased thoracic kyphosis and hip flexion, which lead to a forward displacement of the body’s center of gravity, with knee flexion and ankle plantar flexion as compensation to control balance. Only one author reported worsening of functional balance in subjects with AS. All assessment methods used were considered capable of measuring balance, and no specific scale for patients with AS exists.

Keywords: ankylosing spondylitis, postural balance, posture.

INTRODUCTION

Ankylosing spondylitis (AS) is a chronic inflammatory disease characterized by inflammation of the axial skeleton and by entheses, causing pain, stiffness, and occasionally progressing to joint ankylosis.1–4 Postural control is defined as the capacity to maintain an appropriate relation between the body segments and the environment, adjusting the body to gravity and the task performed, in addition to properly positioning the center of mass in relation to the support base.5

To achieve postural stability, several systems, such as the vestibular, visual, somatosensory, and musculoskeletal systems, are used.7 In the individual with AS, those systems are sound, except for the musculoskeletal one, which is the effector portion related to the motor responses of postural control. The patient’s joints are restricted, with muscle shortening and atrophy, leading to a reduction in the range of motion and flexibility, pain, in addition to deficient balance responses and strategies.6,7

Thus, individuals with AS have a deficient postural control, with reactions of trunk adjustment restricted by the lack of mobility, altered ankle, hip and step postural control strategies, and altered muscle activation patterns. The reduction in the range of motion of the head and neck also affects postural adjustments, hindering gaze stability.8

Reviewing the literature about postural control in individuals with AS is extremely important, because it allows more effective and realistic interventions in such individuals. Thus, this review study aimed at assessing the musculoskeletal alterations in individuals with AS and their repercussions on postural control, in addition to assessing the scales used for that purpose.
METHODS
A bibliographic review was performed in the Bireme and EBSCO HOTS databases and in the PubMed site with the following keywords: “ankylosing spondylitis”, “ankylosing spondylitis balance”, “ankylosing spondylitis postural control”, and “ankylosing spondylitis postural stability”. Articles involving human beings, assessing the postural control and balance of individuals with AS, written in English or Portuguese, and published between 1999 and 2010, were selected. The articles were analyzed according to the type of study, the case series, the postural analysis method used, the postural shifts found, and the respective changes in postural control.

RESULTS
Only four articles met the inclusion criteria established (Table 1). All of them involved field research, but the ways of analyzing postural control and balance differed, hindering the data analysis as a whole. To assess balance, the instruments used were the Berg Balance Scale (BBS), the study of the center of gravity displacements with force platform, and the Bath Ankylosing Spondylitis Metrology Index (BASMI). The major postural disorder found was an increase in thoracic kyphosis and hip flexion, leading to the forward displacement of the body’s center of gravity. Because of that position, compensation of knee flexion and ankle plantar flexion occurred to maintain balance.

Table 1
Summary of the articles found that met the study requirements

<table>
<thead>
<tr>
<th>Souza et al., 2008</th>
<th>Bot et al., 1999</th>
<th>Aydog et al., 2006</th>
<th>Murray et al., 2000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Objective</td>
<td>To assess balance and to correlate it with pain and quality of life</td>
<td>To assess the mechanism of compensation of trunk forward displacement</td>
<td>To assess the existence of deficient dynamic balance and the relation between balance and impairment</td>
</tr>
<tr>
<td>Cohort</td>
<td>30 AS 30 control</td>
<td>4 AS 18 control</td>
<td>70 AS 35 control</td>
</tr>
<tr>
<td>Type of study</td>
<td>Cross-sectional, controlled, cohort; field research</td>
<td>Field research</td>
<td>Field research</td>
</tr>
<tr>
<td>Method of analysis</td>
<td>BBS, VAS, SF-36</td>
<td>Force plate</td>
<td>BASMI, BSS</td>
</tr>
<tr>
<td>Postural displacements</td>
<td>Pelvis retroversion</td>
<td>Hip flexion while standing; progressive kyphosis with forward displacement of the trunk CG</td>
<td>Thoracic kyphosis; forward displacement and lowering of the CG</td>
</tr>
<tr>
<td>Postural control changes</td>
<td>Worse functional balance of individuals with AS</td>
<td>Compensation: knee flexion and/or ankle plantar flexion</td>
<td>No deficient balance was found in the AS group. Compensation: knees and ankles</td>
</tr>
</tbody>
</table>


Because of those postural changes, two authors have found worse balance in individuals with AS, and one of them has reported a correlation between balance and pain. The authors have not ruled out the hypothesis that, with disease progression, the patients could not compensate the postural changes that lead to imbalance.

DISCUSSION
The studies assessed were analytical and cross-sectional, considered adequate for the questions proposed. The studies had significant sampling of the experimental and control groups, ranging from 30–70 individuals, except for that by Bot et al., which assessed only four individuals with AS and 18 controls.

The studies by Souza et al., Bot et al., and Murray et al. have not reported some important data, such as the exact time the patient had to remain in the required posture, the position of the shoulders when measuring the occipital-wall distance and that of the arms when measuring chest expansion, whether there was an interval between the tests, and at which day time the assessment was performed. The interval between assessments, aiming at eliminating the effect of muscle fatigue, and the day time of the assessment, because of morning stiffness, should be controlled because they can influence the results.

Swinkels et al. have suggested some hypotheses to explain the postural changes in AS. In addition to mobility limitation, antalgic position in response to sacroiliac or vertebral joint inflammation and muscle weakness due to a possible primary
denervation process might be related. Murray et al. have also considered the adoption of an antalgic position (the forward trunk inclination) as a possible trigger for postural changes. According to those authors, the forward flexion shortens the soft tissues and increases calcification of the entheses, establishing postural changes. The characteristic changes in patients with AS include straightening of the lumbar lordosis, accentuation of the thoracic kyphosis, and head protraction, manifesting as an increase in upper cervical extension and lower cervical flexion, in accordance with the findings of the studies assessed. 

Souza et al. have reported worse balance in individuals with AS as compared with that of healthy individuals, in addition to a positive correlation between pain (assessed by use of the pain Visual Analogue Scale) and balance (assessed by use of the BBS). Aydog et al. have reported no balance deficiency, but a positive correlation between the occipital-wall distance (BASMI) and the mediolateral stability measure assessed by use of the Biodex Stability System (BSS). A high occipital-wall distance indicates deficient balance – thus, a disorder in dynamic balance can actually occur in AS.

The results by Bot et al. have shown that individuals with AS can hardly extend their hips when in the standing position, maintaining that joint flexed. This increases imbalance, because it causes forward displacement of the center of gravity. The study by Van Royen et al. has assessed, by use of sagittal vertical axis radiography, individuals with vertebral deformities correlating postural changes and compensations to maintain balance. The increase in hip extension is used to compensate the forward displacement of the trunk’s center of mass, thus maintaining balance in the standing position. As deformities progress, the compensation generated by the hips is no longer effective.

According to Aydog et al., other compensations found are knee flexion and ankle plantar flexion. Due to musculoskeletal changes, individuals with AS do not use the hip strategy, requiring the other two strategies as compensatory adjustments to avoid falling when the projection of the center of gravity is outside the supporting base. Disease progression changes postural stability and compensations, mainly aimed at maintaining functionality and adaptation to daily activities.

Murray et al., studying patients with AS, have assessed their static balance with eyes open and eyes closed and compared the results with those of normal individuals. In addition, the modified Schober’s test, occipital-wall distance, and chest expansion test were used. The values achieved by the group with AS were below the normal limit with eyes open and eyes closed. Those results can indicate that patients with AS might have proprioceptive deficits due to the pathology.

The same has been proposed by Swinkels et al., who have reported that the proprioceptive deficit leads individuals with AS to impaired balance. According to those authors, the possible proprioceptive deficit results from the pathologic involvement of the spinal entheses, characteristic of patients with AS. The entheses contain proprioceptive afferents, leading to a change in the spinal position sense. That hypothesis has been verified by an electromagnetic movement analysis system, which measured the accuracy of those subjects in repositioning their spines in the standing and flexed positions. No significant changes in the spinal position sense were found in patients with mild to moderate disease. The researchers have suggested that other proprioceptors might have compensated the affected proprioceptors, because recent research has shown that the capacity of conscious perception of posture and movement depends basically on the information provided by the muscle fuses and Golgi tendon organs, the other receptors playing a secondary role.

Butler et al. have reported that the decrease in proprioception might be related to muscle weakness of the lower limbs, the segment that represents the major source of sensory input used to detect body sways. For that, the researchers have measured the muscle strength of the lower limbs, by using a torquemeter, in three groups as follows: subjects with post-poliomyelitis syndrome, 60–69 year-old women, and women over 70 years. Subjects with significant weakness, even detecting instability, cannot generate an adequate torque in the ankle muscles to correct imbalance. Thus, they compensate increasing the muscle contraction status, which might affect proprioceptive accuracy.

One can suppose that the association between enthesopathy, which affects the afferent proprioceptors, and muscle weakness can justify the deficient balance of individuals with AS. In addition, Murray et al. have emphasized that, in the aging process, a reduction in balance and loss of mobility occur. The aging process causes a decline in the functional quality of all systems participating in postural control, mainly in sedentary individuals, who also experience a reduction in the muscle strength of their lower limbs.

The articles discussed here used methods that assessed only the anticipatory component of balance. The BBS assesses the static and dynamic balances by using usual tasks, such as reach, standing position, and transferences. The BSS assesses static balance by using a mobile balance platform that provides up to 20° of inclination on a 360° surface. Magnometry assesses...
static balance by measuring the movement of the hips on the horizontal plane, by using electromagnetic transmitters and coil receptors. The authors consider that the methods used can measure the balance of individuals with AS, but no specific scale to measure balance in that population has been developed. Regarding the assessment of the compensatory adjustments, the existing scales of easy clinical applicability are scarce. Of the methods related to the assessment of compensatory postural adjustments, dynamic posturography, postural stress test, and sternal nudge test stand out, although, so far, no studies of such instruments in individuals with AS exist.

CONCLUSION

The major postural changes found in patients with AS were accentuation of thoracic kyphosis with forward displacement and lowering of the center of gravity, and hip flexion, which causes the compensations in knee flexion and ankle plantar flexion. The assessment methods used were considered capable of measuring static and anticipatory balance, but no specific scale has been found to assess the balance of individuals with AS. The population with AS has been rarely studied regarding postural control, both dynamic and static. Further studies are required to establish the actual status of postural control in that population.
há diminuição do equilíbrio e perda da mobilidade. O aumento da idade acarreta declínio na qualidade da função de todos os sistemas que compõem o controle postural, principalmente nos indivíduos sedentários nos quais também associa-se uma redução da força muscular nos membros inferiores.\textsuperscript{16}

Os artigos aqui apresentados utilizaram métodos para avaliação que analisaram apenas o componente antecipatório do equilíbrio. A escala de EEB avalia os equilíbrios estático e dinâmico por meio de tarefas habituais, como alcance, bipedestação e transferências.\textsuperscript{6} O BSS avalia o equilíbrio estático por meio de uma plataforma de equilíbrio móvel que proporciona até 20\degree de inclinação em uma superfície de 360\degree.\textsuperscript{8} A magnometria verifica o equilíbrio estático pela mensuração da movimentação dos quadris no plano horizontal, por meio de transmissores eletromagnéticos e receptores de bobinas.\textsuperscript{9}

Os autores consideraram os métodos de avaliação utilizados como capazes de mensurar apenas o equilíbrio de indivíduos com EA, porém não há ainda uma escala específica para mensuração do equilíbrio nessa população. Quanto à avaliação dos ajustes compensatórios, são poucas as escalas existentes e de fácil aplicabilidade clínica. Dentre os métodos relacionados com a avaliação dos ajustes posturais compensatórios, destacam-se a Posturografia Dinâmica,\textsuperscript{17} o Postural Stress Test\textsuperscript{18} e o Sternal Nudge Test,\textsuperscript{19} não havendo, porém, até o momento, estudos desses instrumentos em indivíduos com EA.

CONCLUSÃO

As principais alterações posturais encontradas em pacientes com EA foram acentuação da cifose torácica com anteriorização do exão do tornozelo. Os métodos de avaliação utilizados foram considerados capazes de mensurar o equilíbrio estático e antecipatório, porém não foi encontrada uma escala específica para avaliar o equilíbrio desses indivíduos.

A população com EA é pouco estudada no quesito controle postural, tanto dinâmico quanto estático. São necessários mais estudos para estabelecer o real estado do controle postural nessa população.

REFERENCES


Autoimmune thyroid disease in patients with rheumatic diseases
Teresa Cristina Martins Vicente Robazzi1, Luis Fernando Fernandes Adan2

ABSTRACT
Thyroid function abnormalities and thyroid autoantibodies have been frequently described in patients with rheumatologic autoimmune diseases, such as Sjögren’s syndrome, rheumatoid arthritis, systemic lupus erythematosus and scleroderma. Limited data are available regarding the prevalence and clinical characteristics of autoimmune thyroiditis in other rheumatologic disorders, such as rheumatic fever and juvenile systemic lupus erythematosus. The authors review the association of endocrine autoimmune and rheumatic autoimmune diseases, assessing various age groups and clinical conditions. The bibliographic survey was conducted through the search for scientific articles indexed in the general health sciences databases, such as Latin American and Caribbean Health Sciences Literature (LILACS), Medline/PubMed, and Scientific Electronic Library Online (SciELO). The following descriptors were used: “rheumatic autoimmune diseases and autoimmune thyroid diseases”; “thyroid disorders and rheumatic diseases”; “thyroiditis and rheumatic diseases”; “autoimmune diseases and thyroid”; and “pediatric rheumatic diseases and autoimmune thyroid diseases”. This study showed that, despite contradictory results in the literature, there is a greater prevalence of the association between autoimmune thyroid diseases and rheumatic diseases, highlighting the possibility of common pathogenic mechanisms among them.

Keywords: rheumatic diseases, child, adult, autoimmune thyroiditis.

INTRODUCTION
Autoimmune diseases (AID) are divided into organ-specific and non-specific diseases.1 Autoimmune thyroid diseases (AITD) are considered organ-specific, being represented by Graves’ disease and Hashimoto’s thyroiditis (HT) or chronic autoimmune thyroiditis (CAT). The most common AITD is CAT, considered the prototype of organ-specific AID, characterized by diffuse lymphocytic infiltration of the thyroid gland, presence of anti-thyroglobulin antibodies (anti-Tg) and anti-thyroid peroxidase antibodies (anti-TPO), and endocrine abnormalities ranging from hypothyroidism to myxedema.1,2

Although specific for AITD, the anti-Tg and anti-TPO antibodies have been reported in many patients with non-thyroid diseases, and even in the healthy population.1,2 On the other hand, a high prevalence of autoantibodies directed against specific non-thyroid antigens has been described in patients with AITD, such as antinuclear antibodies (ANA) in HEP-2 cells, whose clinical meaning is unknown14 and whose positivity varies from 9%–35%,4,5 reaching 75% and 69% in anti-TPO and anti-Tg positive patients, respectively.2 An organ-specific and non-specific polyclonal immune response is likely to exist in patients with AITD.4,5

Abnormalities in thyroid function and the presence of thyroid autoantibodies have been frequently described in patients with rheumatologic diseases, with different results according to different authors (Table 1). This study aimed at reviewing the association of endocrine and rheumatic autoimmune diseases, assessing different age groups and clinical conditions.
THYROID AND RHEUMATIC AUTOIMMUNE DISEASES

Sjögren syndrome

The most often reported association of endocrine and rheumatic autoimmune diseases is that between Sjögren Syndrome (SS) and AITD, mainly in adult women, positive for anti-thyroid (ATA) and anti-parietal cell antibodies, suggesting the presence of common environmental and genetic factors, with similar pathogenic mechanisms. The participation of the histocompatibility antigens (HLA) of the haplotypes HLA-B8 and DR3 in both AITD and primary SS (pSS) has been suggested, because of the high frequency of those haplotypes in Caucasian patients with those diseases.1,6–9

The lacrimal, salivary and thyroid glands are very similar from the histological and functional viewpoints, and are greatly susceptible to immune damage. The histopathological lesions of thyroid and salivary glands evidence focal or diffuse infiltration of T lymphocytes, suggesting the same autoimmune response directed to the thyroid follicular cells and the salivary gland epithelium, respectively.8,9 Hansen et al.10 have found five cases of focal autoimmune sialadenitis in 19 patients with AITD, similarly to that which has been shown in patients with primary biliary cirrhosis. Thus, sometimes it can be difficult to clearly establish whether the salivary and eye involvement of SS represents an extrathyroidal manifestation of AITD or, inversely, whether that is an extra-exocrine manifestation of SS.

A retrospective study involving 218 patients with AITD has reported the occurrence of AIDs in 13.7% of their cases, of which the most frequent were SS and systemic lupus erythematosus (SLE).11

Primary SS is ten times more frequent in patients with AITD, and HT is nine times more frequent in patients with pSS as compared with the general population. The major cause of thyroid disease in pSS is HT, and the most frequent hormonal change is hypothyroidism.8

Regarding pSS, hypothyroidism and thyrotoxicosis were found in 14% and 1.8% of the patients, respectively.12 In another study with 479 patients with SS, the frequency of HT found was greater than that in the general population, 6.26% and 1%–2%, respectively, with no increase in frequency, however, for Graves' disease. In addition, symptoms of SS, such as conjunctivitis sicca and xerostomia, have been reported in up to 32% of the patients with HT.13

On the other hand, 10% of the patients with AITD and positive for ANA will be diagnosed with SS, reinforcing once more the possibility of polyclonal autoimmune response to organ-specific and non-specific autoantigens.3

### Table 1

<table>
<thead>
<tr>
<th>Rheumatic disease</th>
<th>Pathophysiological mechanism</th>
<th>Association with AITD</th>
<th>Genetic participation</th>
<th>Major thyroid disease</th>
<th>Most frequent hormone change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sjögren syndrome</td>
<td>Polyclonal autoimmune response1,6–9</td>
<td>+1,8,31</td>
<td>HLA-B8 and DR31,6–9</td>
<td>HT13</td>
<td>Clinical and subclinical hypothyroidism1,17</td>
</tr>
<tr>
<td>SLE</td>
<td>Polyclonal autoimmune response, drugs, low T3 syndrome, chance15,18,21</td>
<td>+17,19</td>
<td>HLA-B8 and DR3 Susceptibility gene in 5q14.3-q1521</td>
<td>HT17,22</td>
<td>Clinical and subclinical hypothyroidism16,22,23</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>Polyclonal autoimmune response1</td>
<td>+15,28–31</td>
<td>HLA-DR3 HLA-DR4 HLA-A2416,18</td>
<td>HT15,28–31</td>
<td>Hypothyroidism11</td>
</tr>
<tr>
<td>JIA</td>
<td>Polyclonal autoimmune response</td>
<td>+43–45</td>
<td>+48</td>
<td>HT14,62–69</td>
<td>Subclinical hypothyroidism43–49</td>
</tr>
<tr>
<td>Scleroderma</td>
<td>Polyclonal autoimmune response, thyroid fibrosis1,39</td>
<td>+39,42</td>
<td>HLA-DR1541</td>
<td>HT11,43</td>
<td>Hypothyroidism10,42–44</td>
</tr>
<tr>
<td>Fibromyalgia</td>
<td>?</td>
<td>?</td>
<td>?</td>
<td>ATA +52</td>
<td>Decrease in thyroid hormones after stimulus with TRH71</td>
</tr>
</tbody>
</table>

SLE: systemic lupus erythematosus; JIA: juvenile idiopathic arthritis; RP: rheumatoid; polymyalgia; GCA: giant cell arteritis; AITD: autoimmune thyroid disease; HT: Hashimoto’s thyroiditis; CRCD: chronic rheumatic cardiac disease; ATA: antithyroid antibodies; TRH: thyrotropin-releasing hormone; +: positive; ?: inconclusive
Thus, most authors tend to screen periodically both the thyroid function in all adult women with SS, even in the absence of symptoms compatible with thyroid disease, and the possible coexistence of SS in all women with AITD, which is justified by the several references in the literature associating SS and AITD.3

Systemic lupus erythematosus

The association between SLE and thyroid dysfunction was first described in 1961 in reports of the association between SLE and HT.14,15 Although the study by Scofield16 has not evidenced greater risk of AITD in patients with SLE, several studies have shown that association.17–19

Although the pathogenic mechanism has remained unknown, genetic influence has been suggested in a study of 35 families with several cases of SLE concomitant with AITD, in which a gene of susceptibility was identified in 5q14.3–q15 (major locus of susceptibility for SLE, also found in AITD). That locus can be shared by patients with SLE and AITD, evidencing a potential genetic link between both diseases.20 Another study has suggested that the presence of HLA-B8 and DR3 is significantly greater in patients with SLE and HT than in the general population.21

The most common thyroid changes in patients with SLE are clinically overt and subclinical hypothyroidism,22,23 estimated in approximately 5.7%, five times more frequent than in the general population.24 The association between SLE and Graves’ disease has been less often described, ranging from 0%–8.9% in different studies, with no increase in prevalence when compared with that of the healthy population.18,22–24

Autoimmunity is one of the several pathogenic mechanisms involved in thyroid dysfunction in SLE – other pathogenic mechanisms include the effect of drugs, such as corticosteroids or immunosuppressors, the effect of the underlying systemic disease (low T3 syndrome or sick euthyroid syndrome), iodine intake, or, simply, chance.17

When assessing the thyroid function of patients with SLE, some interfering factors, such as patient’s age, use of immunosuppressants, and disease activity, should be considered. Acute and chronic systemic diseases have been associated with a significant reduction in total and free T3, a situation known as low T3 syndrome (sick euthyroid syndrome, nonthyroidal illness syndrome), described in patients with several clinical and surgical conditions and after the use of drugs, such as amiodarone, propranolol, non-steroidal anti-inflammatory drugs (NSAIDs), and corticosteroids.17 Corticosteroids slightly inhibit the secretion of thyroid-stimulating hormone (TSH), while NSAIDs interfere with the binding with carrying proteins, reducing the serum concentration of the thyroid hormones.18 The prevalence of that syndrome is controversial, ranging from 0%–47.8%, according to different authors.22

The studies assessing the disease activity in SLE and thyroid dysfunction are not conclusive, and have controversial results.17 However, patients with greater clinical activity and severity of SLE have significant changes in the hypothalamus-pituitary-thyroid axis, even with no evidence of thyroid disease. Few hours after the disease beginning, T3 levels decrease and those of reverse T3 increase, being proportional to disease severity and duration.23 In the adult population, whether the presence of SLE is an independent risk factor for thyroid abnormalities or whether it is a coincidental association is still questioned, since the group at the highest risk for the disease (young women) is also the group at the highest risk for HT.17

However, it is a well-known fact that many signs and symptoms can reflect manifestations of both the thyroid disease and SLE. Because of the frequent association of those diseases, the presence of unspecific symptoms in a patient with SLE should be carefully taken into account, especially when disease activity is low, considering the possibility of an underlying thyroid disease.17

Anti-Tg and anti-TPO antibodies have been more often found in SLE than in the general population, even in patients with no thyroid disease, ranging from 14%–68% in different studies, with increased positivity in those with thyroid dysfunction.17,22

Regarding juvenile SLE, hypothyroidism and ATA have been found in 9% and 20%–34% of the patients, respectively.25 Another study has evidenced anti-Tg in seven of 12 patients with juvenile SLE (58.3%), whose serum levels of TSH, T3 and T4 were normal.21

Rheumatoid arthritis

The association between AIDs, with or without thyroid dysfunction, has also been reported in adults with RA, the pathogenic mechanism being still uncertain.3 The association of the HLA-DR2 and DR4 with the seronegative and seropositive forms of RA, and the greater presence of the HLA-A24, DR3 and DR4 antigens in patients with RA and HT have been evidenced by some authors.24,26

Positivity for the thyroid autoantibodies has been detected in 11% of the patients with RA,27 ranging from 2%–32% in different case series.3,28–31 In a study with 58 patients with RA in United Kingdom families, 6% of the patients had thyroid
diseases and 5% of the men and 15% of the women were anti-TPO positive. In another study with 101 patients with RA of Greece, 12.9% had anti-TPO versus 8.6% of the controls. Similar results have been found in Norway and Canada. Innocenzi et al. have reported positivity for anti-Tg and anti-TPO of 32% and 4%, respectively. El-Sherif et al. have reported an increase in thyroid disorders in patients with RA and/or SLE. Buchanan et al. have demonstrated a statistically significant increase in the association between HT and RA. In addition, Silman et al. have reported high frequency of HT and ATA not only in patients with RA, but also in their families. Deighton et al. have reported a higher prevalence of RA in same-sexed siblings with thyroid diseases as compared with those without thyroid disease.

Although different series have reported an increase in AITD in RA, there is still controversy between the presence of those antibodies and hormone function. Atzeni et al. have reported positivity of 37.1% and 22.9% for anti-TPO and anti-Tg, respectively, subclinical hypothyroidism being present in only 2.8% of the patients. That can be related to the presence of a subclinical thyroiditis or to interactions between free T4 or ATA and other serum factors, such as rheumatoid factor (RF), positive in 65% and 69% of the patients positive for anti-TPO and anti-Tg, respectively.

A study has found a three-fold higher association between thyroid disease (hypothyroidism and HT) and adult women diagnosed with RA, as compared with control women of the same demographic region.

Scleroderma and mixed connective tissue disease

The association between scleroderma and thyroid disease is the only leading to fibrosis of the thyroid gland in the absence of lymphocytic infiltration.

An Italian study has assessed the frequency of ATA and the genetic association with HLA class II antigens in 85 patients with scleroderma. The proportions of patients with anti-Tg and anti-TPO were 12% and 19%, respectively. Individuals with anti-TPO had a higher frequency of the HLA-DR15 allele than patients without those antibodies, suggesting that the HLA-DR15 allele can be a marker of immunogenicity for the formation of anti-TPO.

One case of scleroderma was found in a group of 506 patients with HT and in another of 218 patients with AITD. Kahl et al. in a prospective study have shown that 18 (23%) of 77 clinically euthyroid patients with scleroderma had alterations in their thyroid function tests. In addition, eight (10%) had hypothyroidism, of whom, four had ATA. Such results have confirmed those of the study by Gordon et al., in which 14% and 25% of the patients with scleroderma had severe thyroid fibrosis and hypothyroidism, respectively. In addition, six of seven patients with hypothyroidism (85.7%) had high ATA titers. In the study by De Keyser et al., 39% of patients with clinically stable scleroderma assessed for the presence of thyroid disease, two had hypothyroidism while other seven were euthyroid, but with an exaggerated TSH response to the thyrotropin-releasing hormone, compatible with subclinical hypothyroidism. ATA and thyroid ultrasound were positive in 18% of the patients. Those results indicate an increased frequency of clinically overt and subclinical hypothyroidism in patients with stable scleroderma, which seems to be of autoimmune nature, and has been confirmed by another study carried out in 36 patients with scleroderma.

The association of localized scleroderma or morphea with HT has also been reported, aiming at suggesting that even the localized forms might share an autoimmune pathogenesis. In the mixed connective tissue disease, ATAs have been found in 25% of the patients and clinical hypothyroidism in less than 20%.

Rheumatic polymyalgia and vasculitis

Although rheumatic polymyalgia (RPM) and giant cell arteritis (GCA) have been studied since 1971, there is no definite conclusion about their association with HT. Myklebust et al. and Barrier et al., in prospective studies with 287 and 39 patients, respectively, have reported no association between RPM or GCA and thyroid abnormalities. Wiseman et al., however, studying 367 patients, have reported hypothyroidism in 4.9% of them.

Of the 250 patients with HT, Dent et al. have found seven (2.8%) with RPM or GCA, with a 9.3% prevalence in women over the age of 60 years. Regarding the prevalence of thyroid disease in RPM or in GCA, two prospective controlled studies have excluded any association between them, contrary, thus, to other authors who have reported an increased risk of thyroid disease in those patients.

The largest of those studies has been conducted with 367 patients, 4.9% of whom had hypothyroidism. It is worth noting that, despite the statistically significant results, the population controls of 84 normal participants showed an abnormally low disease rate.

Regarding the association of CAT with other vasculitides, sporadic cases have been reported, and they might not be sufficient to establish a relation between them. Takayasu’s arteritis and IgA-associated vasculitides or Henoch-Schönlein purpura are among the most interesting cases. In that context,
however, special attention should be given to the possibility of false positivity or cross-reactivity induced by the presence of ATA or ANCA in the serum. The cross-reactivity between TPO and myeloperoxidase (MPO) molecules has been studied by Haapala et al. in the sera of six patients with HT and four patients with systemic vasculitis, evidencing that the TPO and MPO molecules contained cross-reaction epitopes exposed in the denatured molecules, which could lead to false-reactivity in solid-phase immunoassays.

In addition, Farsi et al. have reported that some anti-TPO positive sera recognized “normal” MPO, but the sera of most patients with CAT and positive for p-ANCA in human neutrophils also recognized an “abnormal” MPO. On the other hand, a small proportion of MPO-ANCA can react with TPO and be inactivated by heat, providing false-positive results for p-ANCA in human neutrophils fixed in ethanol. Anticardiolipin and antiphospholipid antibodies have also been reported in CAT, but rarely in association with a true syndrome.

Rheumatic fever

In the literature, studies assessing the association between thyroid dysfunction and rheumatic fever, in which all adults have chronic rheumatic cardiac disease (CRCD), are scarce. The first references to the association between rheumatic fever and thyroid dysfunction date back to 1961, with the study of six women with rheumatic heart valve disease, who evolved with thyroiditis, anti-Tg and hyperthyroidism. Since then, few studies have been published; their results are contradictory, either showing no association between rheumatic fever and AITD or evidencing a greater frequency of some type of thyroid dysfunction in patients with CRCD.

A retrospective study with 76 patients with DCRC has evidenced thyrotoxicosis, hypothyroidism and positivity for ATA in the presence of normal thyroid function in nine, three, and seven patients, respectively. More recently, Erdugru et al. have evidenced a greater frequency of HT in patients with rheumatic mitral stenosis (16 of 55; 29%) as compared with their healthy controls. Both studies have suggested the possible existence of an association between CRCD and thyroid disease, which requires, however, further studies.

Juvenile idiopathic arthritis

The association between juvenile idiopathic arthritis (JIA) and HT was first described as a case report in 1968. A new reference in the literature was only made in 1975, with a case report of hypothyroidism secondary to HT associated with diabetic coma in a patient with JIA.

In 1980, Fisher et al. reported the case of a 15-year-old adolescent who had been diagnosed with type 1 diabetes mellitus and HT at the ages of six and nine years, respectively, and who developed clinical findings compatible with polyarticular JIA with positivity for RF and ANA. On the occasion, those authors raised the possibility of some association between the diseases, ruling out the likelihood of chance.

Later, HT was diagnosed in 12 of 27 children diagnosed with JIA (44.4%), most of whom were females (91.7%) with the pauciarticular form of the disease (75%). Of those female patients, 85%, 11.1% and 3.7% had normal thyroid function, compensated hypothyroidism, and thyrotoxicosis, respectively. Anti-Tg was positive in 17 patients (63%) and anti-TPO, in seven (25.9%), with simultaneous elevation of both antibodies in 18.5% of those patients.

In 2001, Koga et al. reported the case of a 17-year-old female adolescent, who had been diagnosed with JIA, pauciarticular form, at the age of six years, being positive for ANA and negative for RF, and who had developed HT with hypothyroidism at the age of 7 years. At the age of 17 years, the patient was diagnosed with autoimmune cholangitis, then progressing to Graves’ disease. The authors emphasized the following physiopathological similarities found in the target organs in HT, in cases of primary biliary cirrhosis, and in the synovial fluid of pauciarticular JIA: high levels of cytokines, such as tumor necrosis factor-α, interleukin-1β, and interleukin-2 soluble receptor.

In 2002, a study with 66 patients with JIA reported the frequency of ATA in nine patients (14%—nine girls, of whom, eight had the pauciarticular form, and one had the polyarticular form) as follows: anti-Tg, in three; anti-TPO, in five; and anti-Tg and anti-TPO in one patient. Three patients showed an echotexture alteration in the thyroid gland parenchyma on ultrasound, being diagnosed with HT (4.5%), a high incidence as compared with that of the general population (1%–2%). Prahalad et al. have reported that at least 12.6% of the relatives of patients with JIA had at least one AID, as compared with 4% of the relatives of controls (P < 0.000001). Of all AIDs, HT was significantly more prevalent in relatives of patients with JIA (P = 0.0008), while the prevalence of other disorders did not significantly differ.

In an Italian study with 151 patients with JIA, 14 (9.3%) had subclinical hypothyroidism (10 females and four males; mean age, 7.4 years, ranging from 2.3–14.9 years). Two patients had HT. Neither clinical nor biochemical hypothyroidism was found in the children with JIA. Seventeen patients (11.9%) were
positive for ATA (16 females; median age, 9.2 years) as follows: positive for anti-TPO, six; positive for anti-Tg, five; and positive for both ATAs, six children. Of all patients, nine (6%) showed a hypoechoic ultrasound pattern compatible with HT.68

A study involving four centers of pediatric rheumatology in Israel with 66 patients with JIA has revealed a higher incidence of ATA (positivity for anti-Tg and anti-TPO of 11.3% and 7.9%, respectively) and subclinical hypothyroidism (12% of the patients) as compared with the normal population. Neither clinically overt hypothyroidism nor symptoms related to the thyroid gland was observed in any patient, and all of them had the pauciarticular form of the disease. The authors have suggested that the following pathogenic mechanisms could be involved in JIA and AITD: immunomodulating effects of the ATAs; molecular mimicry between thyroidal and organ-specific epitopes; and genetic link between thyroidal autoimmunity and susceptibility to the development of JIA.69

On the other hand, a more recent study assessing 80 patients with JIA has evidenced HT in only four (5%), most of whom (three) were females, as follows: systemic onset in one patient, enthesitis-related arthritis in another; and the pauciarticular form in the other two. The status of the thyroid function in those patients was euthyroidism, subclinical hypothyroidism, hypothyroidism and hyperthyroidism, respectively. Contrary to other findings in the literature, neither a case of HT in the pauciarticular form of JIA nor a statistically significant association between JIA and HT was observed. The authors have attributed that to the low frequency of girls (33%) and of the pauciarticular forms of JIA in their study, demographic and clinical characteristics related to HT in the other case series.70

Regarding Graves’ disease, there is only one report of two cases associated with JIA. In the first case, the diagnosis of Graves’ disease preceded the diagnosis of RF-positive polyarticular JIA by 10 years; in the other case, Graves’ disease was diagnosed five years after the onset of psoriatic JIA. Graves’ disease has been known to be an AID associated with the major histocompatibility complex and the T cell inhibitory receptor, CTLA-4. Despite the probable association between JIA and HT, whether a similar genetic relationship exists between Graves’ disease and JIA remains unknown.71

Fibromyalgia
Patients with fibromyalgia (FM) have shown a decrease in the secretion of thyroid hormones two hours after stimulus with TRH, as compared with controls.72 Another study has reported a 20%–24% prevalence of ATA in patients with FM with no evidence of clear thyroid disease, mainly in the elderly and post-menopausal ones.73

CONCLUSION
The development of AITD in the course of rheumatologic AIDs is frequent, although its pathogenesis and clinical significance remain unclear. Regarding pathogenesis, the following hypotheses have been raised: participation of autoantibodies; overlapping of AITD and some AIDs; and systemic inflammatory reaction associated with thyroiditis. Most findings are limited to the occurrence of ATA and subclinical alterations, requiring further studies to assess the clinical impact of thyroid changes in rheumatic patients. Larger studies approaching children are also required, because of the few case series involving that age group, assessing only JIA.
Prahalad et al.\textsuperscript{67} consideraram que pelo menos 12,6% dos parentes dos pacientes com AIJ tinham pelo menos uma DAI, em comparação com 4% dos parentes dos controles ($P < 0,000001$). Entre as várias DAI, a prevalência de TH foi significativamente maior nos parentes de pacientes ($P = 0,0008$), enquanto a prevalência de outros transtornos não foi significativamente diferente.

Em um estudo italiano com 151 pacientes com AIJ, 14 (9,3%) apresentaram hipotireoidismo subclínico (10 mulheres e quatro homens, idade média de 7,4 anos e intervalo de 2,3–14,9 anos). Dois deles apresentaram TH. Não houve casos de hipotireoidismo clínico e bioquímico entre as crianças com AIJ. Dezessete pacientes (11,9%) apresentaram AAT positivos (16 mulheres; idade mediana de 9,2 anos), com anti-TPO em seis, anti-Tg em cinco e ambos em seis crianças. Entre essas, nove (6%) tiveram um padrão ultrassonográfico hipoecogênico, compatível com TH.\textsuperscript{66}

Um estudo envolvendo quatro centros de reumatologia pediátrica de Israel, com 66 pacientes com AIJ, revelou incidência maior de AAT (anti-Tg e anti-TPO positivos em 13,3% e 7,9%, respectivamente) e hipotireoidismo subclínico (12% dos casos) em relação à população normal. Nenhum paciente apresentava hipotireoidismo clinicamente manifesto ou sintomas relacionados à glândula tireoidiana, e todos apresentavam a forma pauciarticular da doença. Os autores sugeriram possíveis mecanismos patogênicos envolvendo AIJ e DAIT: efeitos imunomoduladores dos AAT, mimetismo molecular entre epitópos tireoidianos e órgãos-específicos, e ligação genética entre autoimunidade tireoidiana e suscetibilidade para o desenvolvimento da AIJ.\textsuperscript{66}

Por outro lado, um estudo mais recente avaliando 80 pacientes com AIJ só evidenciou TH em quatro pacientes (5%), dos quais a maioria (três) era do gênero feminino, com tipos de início sistêmico em um deles, artrite relacionada à entesite em outro e poliarticular nos outros dois. O status da função tireoidiana nessas pacientes foi de eutireoidismo, potireoidismo subclínico, hipotireoidismo e hipertireoidismo, respectivamente. Ao contrário dos achados dos demais autores na literatura, não houve nenhum caso de TH nas formas de AIJ pauciarticular, assim como não houve associação estatisticamente significante entre AIJ e TH. Os autores atribuíram esse achado, possivelmente, à baixa frequência de meninas (33%) e de formas de AIJ pauciarticular no estudo, características demográfica e clínica muito relacionadas à TH nas demais séries.\textsuperscript{70}

Em relação à doença de Graves, só há um relato de dois casos associados à AIJ. O diagnóstico de doença de Graves precedeu o diagnóstico de AIJ poliarticular FR positivo em 10 anos no primeiro caso, e foi diagnosticada cinco anos após o início da AIJ psoriásica no outro. A doença de Graves é sabidamente uma DAI, estando associada ao sistema maior de histocompatibilidade e ao receptor inibitório de célula T, CTLA-4. Apesar da provável associação entre AIJ e TH, permanece incerto se uma ligação genética semelhante existe entre doença de Graves e AIJ.\textsuperscript{71}

Fibromialgia

Pacientes com fibromialgia (FM) apresentam diminuição da secreção dos hormônios da tireóide duas horas após estímulo com TRH, quando comparados a controles.\textsuperscript{72} Outro estudo encontrou prevalência de 20%–24% de AAT em pacientes com FM, sem evidência de doença tireoidiana franca, sobretudo entre os pacientes mais idosos e na pós-menopausa.\textsuperscript{73}

CONCLUSÃO

O desenvolvimento de DAIT no curso de DAI reumatológicas é frequente, apesar de permanecerem lacunas obscuras quanto a sua patogênese e a seu significado clínico. Em relação à patogênese, diversas hipóteses são propostas: participação de autoanticorpos, sobreposição entre a DAIT e algumas DAI e reação inflamatória sistêmica associada à tireoidite. A maioria dos achados limita-se à ocorrência de AAT e alterações subclínicas, necessitando de estudos adicionais para avaliar o impacto clínico das alterações tireoidianas nos pacientes reumáticos. Maiores estudos também são necessários no que se refere à faixa etária infantil no primeiro caso, e foi diagnosticada cinco anos após o início da AIJ psoriásica no outro. A doença de Graves é sabidamente uma DAI, estando associada ao sistema maior de histocompatibilidade e ao receptor inibitório de célula T, CTLA-4. Apesar da provável associação entre AIJ e TH, permanece incerto se uma ligação genética semelhante existe entre doença de Graves e AIJ.\textsuperscript{71}

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Ocorrência de doenças autoimunes tireoidianas em pacientes com doenças reumáticas


Cocaine-induced midline destruction lesions with positive ANCA test mimicking Wegener’s granulomatosis

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ABSTRACT
Chronic use of cocaine by inhalation may induce midline destructive lesions (CIMDL), which can sometimes be difficult to distinguish from the ear, nose and throat lesions of Wegener’s Granulomatosis (WG). We describe the case of a 43-year-old female patient admitted with a two-year history of nasal obstruction and rhinorrhea. She had been diagnosed with WG for five months, being on prednisone and cyclophosphamide. On her physical examination, perforation of her nasal septum and palate was observed. Laboratory tests showed elevated acute phase proteins and a positive p-ANCA test. ELISA assays anti-proteinase 3 and myeloperoxidase were negative. The paranasal sinus computed tomography (CT) showed destruction of the nasal septum and palate, in addition to bilateral maxillary sinusitis. Chest CT was normal. Nasal mucosal biopsy revealed an inflammatory infiltrate, with neither granuloma nor vasculitis. When questioned, she admitted being a cocaine user for five years. Medical therapy and cocaine use were withdrawn. She has been followed up for six months and no other lesion or other organ symptoms occurred. Differential diagnosis in patients with midline destructive lesions can be very challenging. Evaluation should include enquiry about intranasal use of cocaine. Although ANCA testing does not clearly differentiate the ANCA found in some patients with CIMDL from those found in WG patients, the localized involvement and the biopsy findings non-characteristic of small vessel granulomatous vasculitis should be recognized as features for cocaine-induced lesions.

Keywords: cocaine, Wegener’s granulomatosis, antineutrophilic cytoplasmic antibody, vasculitis.

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INTRODUCTION
Perforation of the nasal septum or palate is a complication caused by cocaine chronic use.1 This condition can mimic several medical conditions, including: leishmaniasis, syphilis, lymphoma, blastomycosis and Wegener’s granulomatosis (WG).2 WG is a systemic vasculitis characterized by inflammation and necrosis of small blood vessels which more commonly affects upper and lower airway (lung parenchyma and bronchi), as well as kidneys, causing a glomerulonephritis that can evolve to renal failure.3

Cocaine is an alkaloid that increases the activity of monoamine neurotransmitters in the central and peripheral nervous system by blocking reuptake pumps (transporters) of dopamine, norepinephrine and serotonin. This substance can be used orally, intranasally (inhalation), by intravenous or subcutaneous injections, or via genital mucosa, and it also can be smoked (crack). Each one of the routes of administration presents differences in the severity and quality of the effects and risks of complications associated with its use. Inhaled cocaine can cause inflammation and ulceration of the nasal mucosa with perforation of the septum (cocaine-induced...
midline destructive lesions – CIMDL). The mechanism of nasal inflammation and necrosis is multifactorial and includes local ischemic vasoconstrictor effect, local trauma, irritation of the mucosa due to other mixed substances, deficit in mucociliary transport and, rarely, secondary bacterial infection. Frequent and prolonged inhalation can cause osteocartilaginous necrosis which can extend to cornets and maxillary sinus. In rare circumstances, the bones of the palate suffer necrosis and perforate.

Here we describe the case of a patient who presented with cocaine-induced nasal/palate perforation and also with a positive perinuclear antineutrophil cytoplasmic autoantibody (p-ANCA) test. We further discuss the diagnosis work-up and differential diagnosis with limited WG.

CASE REPORT

A 43-year-old female patient was admitted in our hospital with a two year history of nasal obstruction, nasal voice and rhinorrhea. She reported a WG diagnosis five months before admission and had been on oral prednisone 60 mg/day and cyclophosphamide 100 mg/day since then. Her physical examination revealed a nasal septum and palate perforation with 1.5 cm of extension (Figure 1). Laboratory tests showed hematocrit 30.2%, hemoglobin 9.9 g/dL, mean cell volume (MCV) 63.7 fl, leukocytes 8,500 cells/mm³, platelets 521,000/mm³, erythrocyte sedimentation rate (ESR) 120 mm/h, C-reactive protein (CRP) 31.2 mg/L, and creatinine 0.6 mg/dL. Tests for ANA, rheumatoid factor, hepatitis C virus, HIV virus, hepatitis B virus, venereal disease research laboratory (VDRL) and FTA-ABS were all negative. Purified protein derivative (PPD) test was not reactor. Leishmaniasis and blastomycosis serology were both negative. The ANCA test (indirect immunofluorescence – IIF) was positive (1:360 dilution) with a p-ANCA pattern. Enzyme-linked immunosorbent assays (ELISA) for antigen-specific ANCA directed against proteinase 3 (PR3) and myeloperoxidase (MPO) were negative. The nasal parasinus computed tomography (CT) showed destruction of nasal septum and palate, as well as bilateral maxillary sinusitis. Chest CT was normal. A nasal mucosal biopsy revealed an inflammatory infiltrate, without granuloma or vasculitis (Figure 2). When questioned, she admitted to be a cocaine user (nasal inhalation) for at least five years. Prednisone and cyclophosphamide were suspended and a causal relationship between cocaine and the destructive lesions was established. The patient no longer used the drug. She has been followed-up for six months and no other lesion or other organ symptoms occurred.

DISCUSSION

Cocaine use can cause destructive facial injuries that mimics the clinical picture of other diseases associated with necrotizing midfacial lesions. This is an important clinical scenario that is likely to become more common as worldwide use of cocaine increases.

ANCA directed against PR3 or MPO are sensitive and specific markers for the idiopathic small vessel vasculitis including WG. It is generally believed that the presence of a positive ANCA test with either of the two antigen specificities
facilitates the differential diagnosis of vasculitis. The sensitivity of c-ANCA for WG diagnosis ranges from 34%–92%, depending on the extension of the compromise of disease, and the specificity ranges from 88%–100%. The p-ANCA pattern which usually corresponds to the presence of anti-MPO antibodies (in patients with vasculitis) occurs in only 5% of WG patients. When the antigenic target is not the MPO, as occurred in our patient, a multitude of other targets have been reported including cathepsin G, lactoferrin, elastase and lysozyme. The International Consensus Statement on testing and reporting of ANCA states that ANCA are most readily demonstrated by using a combination of IIF of normal peripheral blood neutrophils and ELISAs that detect ANCA specific for PR3 or MPO. According to this consensus, a p-ANCA positive-MPO-ANCA negative result may occur in treated, inactive, or relapsing patients. When the antigenic target is not the MPO, as occurred in our patient, a multitude of other targets have been reported including cathepsin G, lactoferrin, elastase and lysozyme. The International Consensus Statement on testing and reporting of ANCA states that ANCA are most readily demonstrated by using a combination of IIF of normal peripheral blood neutrophils and ELISAs that detect ANCA specific for PR3 or MPO. Consequently, a p-ANCA positive-MPO-negative result may occur in treated, inactive, or relapsing patients. When the antigenic target is not the MPO, as occurred in our patient, a multitude of other targets have been reported including cathepsin G, lactoferrin, elastase and lysozyme. The sensitivity of c-ANCA for WG diagnosis ranges from 34%–92%, depending on the extension of the compromise of disease, and the specificity ranges from 88%–100%. The p-ANCA pattern which usually corresponds to the presence of anti-MPO antibodies (in patients with vasculitis) occurs in only 5% of WG patients. When the antigenic target is not the MPO, as occurred in our patient, a multitude of other targets have been reported including cathepsin G, lactoferrin, elastase and lysozyme.

Instances of positive ANCA test results have also been reported in patients with lesions attributed to cocaine abuse. Trimarchi et al. evaluated 25 patients with sinus and nasal necrosis secondary to cocaine and showed that 14 (56%) of these patients were ANCA positive, being nine with p-ANCA and five with c-ANCA pattern, some of them with reactivity against PR3. In another study, Wiesner et al. evaluated human neutrophil elastase (HNE) ANCA in CIMDL, WG and MPA patients and healthy volunteers. The authors found that 19 patients (76%) with destruction of nasal septum secondary to cocaine had ANCA positive (in most cases p-ANCA) and 12 (57%) had positive reactivity against PR3. Among patients with CIMDL, HNE ANCAs were detectable in 84%. Fifty-seven percent of HNE ANCA-positive CIMDL sera were also PR3 ANCA-positive. Sera obtained from patients with WG or MPA were universally HNE ANCA-negative, as were sera obtained from healthy controls. They concluded that HNE ANCAs may discriminate between CIMDL and WG, whereas a positive test result for PR3 ANCA may not. Unfortunately we could not test for the HNE ANCA in our patient, since until now it is available only for experimental purposes.

As some patients, especially with limited WG, might have a p-ANCA positive-MPO-negative result and drug use history provided by patients is notoriously unreliable, differentiation of cocaine-induced lesions from necrotizing granulomatous inflammation of the upper respiratory tract associated with WG may be very challenging. In addition, specifically in our case, the patient presented with anemia and elevated ESR and CRP which could represent other factors for confusion. We cannot be sure about the reason for these findings but anemia with low VCM is extremely common in fertile women and the inflammatory tests are very unspecific. The mechanism of nasal inflammation and necrosis is multifactorial and also includes secondary bacterial infection. Thus, it is possible that elevated inflammatory tests could be ascertained just for the local inflammation and necrosis. Fortunately, although nasal and sinus biopsies have less than a 30% chance of showing granulomatous inflammation due to the small size of the specimens obtained, certain non-characteristic biopsy findings may alert towards one diagnosis or the other.

Trimarchi et al. evaluated histopathologic examination of 18 cocaine abusers with midline destructive lesions in comparison to 21 WG patients. In summary, biopsies with nonspecific changes were more frequent in CIMDL (44%) than in WG patients (24%), but the difference was not statistically significant. Microabscesses in the vascular wall and perivenulitis were observed with similar frequencies in both groups. Leukocytoclastic vasculitis and fibrinoid necrosis appeared to be more frequent in WG (P = 0.02). However, when the data analysis was based on the occurrence of the lesion in individual patients rather than individual biopsies, no difference was detectable: it occurred in six of 18 CIMDL and in nine of 21 WG patients (P = 0.11). In contrast, extravascular changes consisting of stromal granulomas with giant cells, microabscesses, and deeply located necrosis were features exclusively found in WG (P = 0.001).

In summary, whereas routine ANCA testing does not clearly differentiate the ANCA found in some CIMDL patients from those of WG patients, more detailed investigations suggest interesting differences between the ANCA of the two patient populations. Vascular abnormalities mimicking vasculitis are frequently found in biopsy specimens of CIMDL patients and are not helpful in the differential diagnosis. However, extravascular necrosis, microabscesses, granulomas, and giant cells are differentiating histopathologic hallmarks of WG. A complete evaluation of patients with nasal septum and/or palate perforation should always include an investigation about the use of cocaine, ANCA, and histopathologic examination in order to avoid diagnostic mistakes and possibly harmful treatments.
Lesões destrutivas da linha média induzidas por cocaína com ANCA positivo mimetizando a granulomatose de Wegener

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REFERÊNCIAS
Thoracic myelopathy due to calcification of the ligamentum flavum with hyperproteinorachia and responsive to steroid therapy: case report

Flávia Yuri Shiguematsu¹, Elaine Cristina Caon de Souza¹, Adriana Fontes Zimmermann², Gláucio Ricardo Werner Castro³, Ivânio Alves Pereira⁴, Fabricio Souza Neves⁴

ABSTRACT
Calcification and ossification of the ligamentum flavum or of the posterior longitudinal ligament are causes of compressive myelopathy, more frequent in the lower thoracic levels, and extremely rare in Western populations. Surgical decompression is the only therapy, but the disease is usually progressive, and its recurrence after surgery is common. Inflammatory mediators might play a role in the progression of compressive myelopathy, but, to our knowledge, the therapeutic approach involving anti-inflammatory agents has never been tried before. We report a case of compressive myelopathy due to calcification of the ligamentum flavum, in which hyperproteinorachia and response to steroid therapy have been observed. Those data have not been published before and might provide new ideas for the disease understanding.

Keywords: ligamentum flavum, ossification of the posterior longitudinal ligament, spinal cord compression, cerebrospinal fluid.

INTRODUCTION
Calcification and ossification of the ligamentum flavum (CLF and OLF, respectively) or of the posterior longitudinal ligament (PLL) of the spine are often reported in Far Eastern countries, where they are common causes of compressive myelopathy, usually at the thoracic level. However, that disorder is extremely rare in Western populations, in which only cervical and lumbar degenerative myelopathies or radiculopathies are commonly found.¹ Most of the pathophysiological mechanisms of that condition and reasons for its peculiar prevalence in Eastern countries are unknown. Hypertrophy of the spinal ligaments might begin in response to mechanical stress, but in patients with genetic or systemic predisposing factors, that process progresses with ligament hyperplasia, vascular neoformation, cartilaginous metaplasia, calcification, and, eventually, replacement by compact bone.² The local expression of growth factors, such as bone morphogenetic proteins (BMP)³ and transforming factor-β (TGF-β),⁴ can be a predisposing element. Thus, cytokines might play a role in the progression of calcification and ossification of the spinal canal ligaments, but no therapeutic intervention has been proposed to act on those elements.

We report the case of a female patient with compressive thoracic myelopathy caused by CLF, a rare disease in the Brazilian population. Informed written consent was provided by the patient for the publication of her data and images. Although a previous case has already been reported in the Brazilian literature,¹ ours is particularly relevant, because the initial diagnosis of systemic lupus erythematosus (SLE) led to the observation of hyperproteinorachia and response to steroid therapy. Such findings have never been reported in the medical literature and might contribute to the better understanding of that entity.
CASE REPORT

The patient is a 47-year-old woman who sought the emergency service with spastic paralysis of her lower limbs, which had begun five days before with no history of trauma. On physical examination, she had grade 0 strength in her lower limbs with hyperreflexia and clonus, anesthesia below T10 level, and facial erythematous exanthem, predominating in her malar regions and nasal bridge, but also present in the frontal and perioral regions, with telangiectasia and some small pustules (Figure 1). The patient also had type 2 diabetes mellitus and systemic arterial hypertension, for which she had been using metformin and captopril for five years. Her Body Mass Index was 35. Her complementary exams were performed at the emergency, and neither her vertebral column nor her skull showed abnormalities on computed tomography. Lumbar puncture was performed, and the analysis of the cerebrospinal fluid showed important hyperproteinorachia (784 mg/dL), with mild hypercellularity (10 cells/mm³, lymphocytes and monocytes). The patient was positive for the antinuclear antibody (ANA) with titers of 1/80 and fine speckled pattern. The diagnosis of SLE with inflammatory transverse myelitis was proposed, and pulse therapy with methylprednisolone, 1 g/day for three days, was initiated immediately. Anticoagulation with enoxaparin was also initiated, considering the possibility of ischemic myelopathy as an alternative diagnosis.

A significant improvement was observed, with disappearance of the clonus on the next day and strength recovery. Ten days later, the patient had grade 3 strength in both lower limbs, when a new analysis of the cerebrospinal fluid revealed protein levels almost normal (60 mg/dL). The search for the following elements was negative: oligoclonal bands, HTLV–I, HTLV–II, Schistosoma mansoni, bacteria, mycobacteria, and fungi.

However, except for the facial exanthem and low ANA titers, the patient lacked any other criteria for SLE. The following exams were normal: anti-DNA, anti-Sm, complete blood count, creatinine, urinary sediment, serum complement erythrocyte sedimentation rate, and C-reactive protein. There was no evidence of peripheral neuropathy. The anticardiolipin and lupus anticoagulant antibodies were not found, and the echocardiogram was normal. The skin biopsy identified rosacea, and the diagnosis of SLE was definitely ruled out.

Magnetic resonance imaging (MRI) of her skull was normal, making the diagnosis of multiple sclerosis less likely. However, the MRI of the vertebral column revealed hypertrophy of the ligamentum flavum between the T2–T10 levels, with spinal cord compression (Figure 2). Anticoagulation
DISCUSSION

Calcification of the ligamentum flavum probably represents an initial stage of OLF, both conditions being closely related to those affecting the PLL, calcification and ossification of the PLL (CPLL and OPLL, respectively). Ossification of the PLL has been more extensively investigated.

Both CPLL and OPLL might be multifactorial diseases, in which complex genetic and environmental factors interact. Polymorphisms in the collagen 11A2 and 6A1 genes have been associated with OPLL. The role of collagen VI and collagen XI in that condition is still uncertain, but they are supposed to serve as a framework for chondrocytes to elaborate the ossification process, propitiated by those mutations.

There are two models of OPLL in animals. The tiptoe walking mouse is a natural mutant with extensive heterotopic calcification. He has a single mutation in the nucleotide pyrophosphatase gene, which regulates the physiological calcification of tissues by producing pyrophosphates.3 The Zucker rat, an animal model of obesity, dyslipidemia, and hyperinsulinemia, also develops ossification of the spinal ligaments.

The local expression of cytokines and growth factors has also been reported in the OPLL. The BMP-2 and TGF-β have been more intensively investigated, being present in the tissue matrix adjacent to the ossified ligaments.4 Thus, the major hypothesis for the pathophysiology of OPLL is that mechanical stress is converted into a biological response that induces adequate production of specific cytokines that act as growth factors, leading to calcification and ossification of pathologically predisposed collagen fibers of the spinal ligaments.5

In most cases reported, totally developed ossification of the ligament has been found at the time of diagnosis. However, there are transitory stages between the normal ligament and its complete ossification. Hypertrophy of the PLL (HPLL) is considered to be the precursor of the OPLL. Mizuno et al.2 have suggested that HPLL is replaced by lamellar bone in an individual with HPLL due to a mechanical degenerative process, in the presence of genetic factors that propitiate the appearance of secondary calcification and progressive ossification. In HPLL histological studies, ligament hyperplasia and metaplasia have been described, as well as focal areas of calcification, where depositions of calcium pyrophosphate dihydrate and calcium hydroxyapatite are commonly found.6 Those findings support the hypothesis that OPLL is a progressive disease with different stages, described as “OPLL in evolution” by Epstein.7 This disease probably progresses with ligament hypertrophy, vascular neoformation, cartilaginous metaplasia of the ligaments, calcification with crystal deposits, and, eventually, ossification of the ligaments inside the spinal canal. Briefly, Okada et al.4 have reported that, initially, the ligament undergoes hypertrophy and calcification, and, then ossifies.

For didactic purposes, those four conditions (OPLL, CPLL, OLF and CLF) could be gathered under the same group denomination as “disease of the spinal canal ligaments” (DSCL). Those conditions are a frequent cause of compressive myelopathy in Far Eastern countries. Its prevalence reaches 3.6% in South Korea8 and 2.8% in Taiwan.9 They affect mainly the lower thoracic levels of the spinal cord, causing progressive spastic paraplegia. Surgical decompression is the only therapy, and the shorter the time until surgery, the better the neurological prognosis.10 In the largest epidemiological study about thoracic myelopathy carried out in Japan,11 OLF accounted for 56% of the cases, followed by OPLL (11%) or the association of both conditions (9%). Disc herniations (11%) or osteophytes (8%) were less common causes of spinal compression at that level.12 Patients with several metabolic disorders, such as hypoparathyroidism, hypophosphatemic rickets, and type 2 diabetes mellitus, have a higher prevalence of DSCL than the general population. Recent studies have been concentrated on the fact that obesity and type 2 diabetes mellitus are independent risk factors for the appearance of DSCL, probably due to hyperinsulinemia.5

Regarding correlated disorders, diffuse idiopathic skeletal hyperostosis (DISH), unlike DSCL, is more common in Caucasians and rare in the Eastern populations. It consists in ossification of the anterior longitudinal ligament of the vertebral column, outside the spinal canal. Despite those differences, DISH shares with DSCL two risk factors: obesity and diabetes mellitus. On the other hand, it is worth emphasizing that the diseases that affect the PLL and the ligamentum flavum occur preferentially in the same population (Asians), share their risk factors and have similar histological and histochemical findings, being considered parts of the same spectrum.5
Because our patient had no ossification on biopsy, one could question whether the calcium deposits found could be attributed to another deposition disease. In fact, some authors have reported cases of myelopathy caused by calcium pyrophosphate deposition disease (CPDD). However, calcium pyrophosphate deposition in spinal ligaments, in most cases, could also be attributed to the metaplasia and calcification observed in DSCL. Under such conditions, chondrocytes produce inorganic pyrophosphate that binds to ionized calcium and deposits on collagen fibers. Focal depositions of calcium pyrophosphate along the hypertrophied ligament are commonly found in DSCL. It is worth noting that nucleotide pyrophosphatase dysfunction might play a role in the DSCL pathogenesis. In a review of 25 cases of compressive myelopathy attributed to CPDD reported in the literature, only nine (35%) evidenced peripheral arthritis attributed to CPDD. However, most of the remaining cases could be true examples of calcium pyrophosphate deposition in spinal ligaments due to DSCL, instead of true CPDD.

Our patient showed neither clinical nor radiological evidence of CPDD. She had obesity and type 2 diabetes mellitus, both risk factors for DSCL, and the biopsy revealed important hypertrophy of the ligamentum flavum, with focal deposition of calcium on the areas of cartilaginous metaplasia. Those findings are strong evidence that DSCL is the major cause of calcium deposition in hypertrophied ligaments with metaplasia, and not that the calcium deposition is the initial cause of the ligament disease. However, DSCL seems to be very rare in Western countries, where thoracic compressive myelopathy is much less common than the degenerative stenoses of the cervical or lumbar spinal canal. In our patient, DSCL was not even suspected in the initial approach, and the presumptive diagnosis of SLE led us to perform cerebrospinal fluid analysis and steroid therapy, which significantly improved her symptoms. This led us to intriguing observations.

To our best knowledge, this is the first report about abnormality of the cerebrospinal fluid in a patient with DSCL, although dura mater involvement has been reported in such cases. Significant hyperproteinorachia suggests the inflammatory process relevance in the DSCL pathogenesis. Some inflammatory mediators might act as growth factors for the spinal ligaments, leading to ossification. In addition, this is apparently the first description of a therapeutic approach for myelopathy due to DSCL, including high dosage steroid therapy prior to surgical decompression.

In our patient, laminectomy was performed two weeks after symptom onset, representing a significant delay. Nevertheless, an excellent result was obtained. In addition, our patient had an acute onset of compressive symptoms, which is considered a sign of poor prognosis. In the series by Fong et al., from Singapore, patients with acute myelopathy had persistent symptoms, with ambulatory disability despite surgical decompression and rehabilitation programs. Thus, we believe that steroid therapy might have acted as a “bridge” therapy, allowing relief from the spinal compression until the definite surgical decompression. Our report supports the use of corticosteroids in cases of compressive myelopathy due to DSCL, when neurosurgery is not available.

In addition, although DSCL improves after surgery, it usually has a long-term progression. In a review of eight studies, Inamasu et al. have reported radiological progression in 36%–86%, with clinical recurrence of compressive myelopathy in 2%–53% of the cases. In face of that, one might ask whether long-term anti-inflammatory therapy in the post-operative period could prevent disease recurrence and which patients could benefit from that.

Finally, we could ask whether our patient’s pustular dermatosis was somehow related to the triggering of DSCL. One case of the association of the SAPHO (synovitis, acne, pustulosis, hyperostosis and osteitis) syndrome and DSCL has already been reported. Ours is the first report of the association of DSCL and rosacea. The small experience of a single case does not allow any definitive conclusion, but one might hypothesize that patients with pustular dermatosis and DSCL could represent a special group, in which inflammatory mediators would be the major cause of ligament hypertrophy and its progressive ossification.

We suggest that some cases of DSCL leading to thoracic myelopathy depend on inflammatory phenomena, being, thus, responsive to steroid therapy. That therapy might be useful when surgical decompression cannot be performed immediately, since the final prognosis depends on the total duration of the symptoms associated with spinal compression. One relationship between DSCL and pustular dermatosis can be hypothesized, but, due to the scarcity of such cases in Brazil, further studies are required in countries where such condition is more frequently found.
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Cutaneous leishmaniasis in a patient with ankylosing spondylitis using adalimumab

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ABSTRACT

Leishmaniasis is an anthropozoonosis caused by species of Leishmania and can have different clinical presentations, depending on the parasite-host relationship. Tumor necrosis factor-α (TNF-α) is a cytokine essential to infection control, especially against intracellular parasites such as Leishmania. Anti-TNF-α strategies have had a marked impact on the treatment of rheumatic diseases, but the clinical use of those antagonists has been accompanied by an increased report of infections. We report the first case of cutaneous leishmaniasis in a patient with ankylosing spondylitis treated with adalimumab and methotrexate in Brazil. We believe that, in this case, there was no association between the anti-TNF-α treatment and cutaneous leishmaniasis, because the disease was limited to only one ulcer that healed completely after treatment. More studies, however, are necessary to better understand the possible relationship between anti-TNF-α agents and leishmaniasis.

Keywords: ankylosing spondylitis, tumor necrosis factor-α, cutaneous leishmaniasis, methotrexate.

INTRODUCTION

Leishmaniasis is an anthropozoonosis that can be caused by several flagellated protozoa species of the Leishmania genus, transmitted by insects of the Lutzomyia genus. Those protozoa are obligate intracellular parasites of mononuclear phagocytic system cells.1,2 The disease is considered a public health problem in 88 countries, with 1–1.5 million new cases of American tegumentary leishmaniasis (ATL) registered every year.1 More than 90% of the cases of ATL occur in six countries, including Brazil.2 The clinical manifestation of the disease depends not only on the Leishmania species involved, but also on the patient’s immune status, a wide spectrum of clinical forms existing depending on the host’s cellular immune response.1

The tumor necrosis factor-α (TNF-α) is a pro-inflammatory cytokine produced by macrophages, involved in both the pathogenesis of several inflammatory diseases and the immune-mediated response to several infections, especially that against intracellular pathogens.1 The TNF-α is essential in the resistance to several microorganisms, such as the infectious species of Leishmania.1,4

Since the beginning of the commercialization of the anti-TNF-α biologics, at the end of the 1990s, the use of those drugs has become increasingly frequent. Their efficacy in the management of several immune diseases, such as rheumatoid arthritis, spondyloarthritides, Crohn disease and psoriasis, has confirmed their use.2,5,6 The anti-TNF-α therapy, however, is affected by the increasing number of opportunistic infections, such as Pneumocystis jirovecii pneumonia, histoplasmosis, cytomegalovirus, aspergillosis, cryptococcal meningitis, leishmaniasis, and mainly tuberculosis.3,5 We report one case of ATL in a female patient with ankylosing spondylitis and receiving the anti-TNF-α therapy with adalimumab (ADA).
**CASE REPORT**

The patient is a 36-year-old female complaining of an ulcerated, painless, and non-pruriginous lesion on her right leg for two months, which appeared after a trip to Timon, an important endemic area of tegumentary leishmaniasis in the state of Maranhão, Brazil. She had no other clinical complaints. The patient had ankylosing spondylitis and had been on ADA, 40 mg every 21 days, and methotrexate (MTX), 10 mg/week, for one year. On physical examination, an ulcer was observed on the lateral face of her right leg, with erythematous, elevated and well-defined margins, granulomatous base, and measuring 1.5 cm of diameter (Figure 1). The rest of her physical examination showed no other changes.

Her tuberculin skin test showed non-reactive purified protein derivative, and the Montenegro test evidenced a 12-mm reaction (reactions greater than 5 mm are considered positive). The direct search for Koch’s bacillus and Leishmania, as well as the cultures for mycobacteria and fungi, were negative. The histopathology of the lesion showed exuberant granulation tissue. The other exams, such as electrocardiography, echocardiography, blood cell count, and renal and hepatic functions, showed no changes.

Based on the epidemiology, the aspect of the ulcer and the positive Montenegro test, the diagnosis of ATL was established. The use of ADA and MTX was suspended, and pentavalent antimony (Glucantime®) started. After 30 days of use, complete regression of the lesion occurred, leaving an atrophic scar on the site.

**DISCUSSION**

TNF-α is a cytokine with an important role in the host defense against Leishmania species infection. Those protozoans are obligate intracellular parasites of macrophages, and the infection control requires the activation of those cells and formation of granulomas.1,2,7,8 Most pathogens induce a rapid increase in the production of the cytokine in a host’s attempt to control infection. The anti-TNF therapy impairs that initial response, increasing susceptibility and reducing the ability to fight infections such as leishmaniasis.3 In a study with mice infected with Leishmania major, the presence of anti-TNF antibodies was related to both an important reduction in the leishmanicidal activity of macrophages, and the development of larger cutaneous lesions.7 TNF is implicated not only in inducing the formation of granulomas, but also in maintaining them, which can explain the participation of the anti-TNF-α therapy in the reactivation of granulomatous diseases.8

The cases of cutaneous leishmaniasis in patients on biologics reported in the literature are few. A search in the PubMed/MEDLINE database evidenced six cases of ATL related to that therapy: three patients were on infliximab (IFX)4,6 and the other three were on ADA.8–10 No article relating etanercept (ETN) to that clinical presentation of leishmaniasis has been found. The association with the visceral form, however, has been more widely reported.4

Considering the important role of TNF-α in the body’s defenses, the major adverse effects expected in patients receiving anti-TNF therapy are infections.6 Several randomized clinical trials have concluded that the use of agents such as IFX and ADA results in an increased risk for infections.5 The risk of developing opportunistic infections seems to be greater in the first year of treatment, especially in the first months,3,5 with a tendency towards more infectious complications in patients using IFX as compared with those on either ADA or ETN.5 Atypical manifestations, disseminated forms and paradoxical reactions to treatment are the most common clinical presentations of opportunistic infections in those patients.3

Despite the use of those drugs, our patient developed response to the Montenegro test and responded well to the treatment with Glucantime, having neither disseminated nor relapsing cutaneous lesions – that is, apparently, those drugs have not changed the natural course of the disease. Healthy individuals can also develop ATL when traveling to endemic areas. Thus, we believe that, in the present case, there was no association between the use of the anti-TNF therapy and the appearance of the ulcer. New studies, however, are required to better clarify the existence of that relationship.
Unfortunately, the vaccination against leishmaniasis is not systematic, although effective vaccines have been reported.\textsuperscript{11-13} Thus, general measures are recommended when traveling to endemic areas, such as the use of repellents and clothes covering larger areas of skin, avoiding exposure during the times of the vector’s activity (twilight and night).\textsuperscript{1}

Although a positive epidemiology does not contraindicate the use of drugs such as ADA, a strict follow-up should be maintained in those cases. So far, there has not been enough evidence to support the search for leishmaniasis in the screening for the treatment with biologics.
e o aparecimento da úlcera. Novos estudos, entretanto, são
necessários para esclarecer melhor a existência de relação.

Infelizmente, não há formas sistemáticas de vacinação
contra a leishmaniose, apesar de haver relatos de vacinas
eficazes.\textsuperscript{11–13} Então, recomendam-se medidas gerais ao viajar
para zonas endêmicas, como o uso de repelentes e de roupas
mais cobertas, evitando exposição no horário de atividade do
vetor (crepúsculo e noite).\textsuperscript{1}

Embora a presença de epidemiologia positiva não contrain-
dique o uso de medicamentos como o ADA, um acompanha-
mento rigoroso deve ser mantido nesses casos. Até o momento,
ninguem evidências suficientes que torne necessária a
pesquisa de leishmaniose como \textit{screening} para o tratamento
com medicação biológica.

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Influence of creatine supplementation on bone mass of spontaneously hypertensive rats

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ABSTRACT

Introduction: Recent evidence has suggested that creatine supplementation (Cr) can increase the bone mineral density (BMD) of the femur in healthy growing rats. Nevertheless, studies assessing the efficacy of the Cr supplementation in conditions characterized by bone mass loss are scarce. Objective: To investigate the effect of Cr supplementation on BMD and bone mineral content (BMC) in spontaneously hypertensive rats (SHRs), an experimental model of osteoporosis. Materials and methods: Sixteen 8-month-old male SHRs were randomly allocated into two groups matched by body weight: 1) Pl group: SHRs treated with placebo (distilled water; n = 8); and 2) Cr group: SHRs treated with Cr (n = 8). After nine weeks of supplementation, the animals were euthanized and their femur and spine (L1-L4) were analyzed by use of densitometry (Dual Energy X-Ray Absorptiometry). Results: No significant difference was observed between the groups regarding either the spine or the total femur measures as follows: spine – BMD (Pl = 0.249 ± 0.003 g/cm² vs. Cr = 0.249 ± 0.004 g/cm²; P = 0.95) and BMC (Pl = 0.509 ± 0.150 g vs. Cr = 0.509 ± 0.017 g; P > 0.99); and total femur – BMD (Pl = 0.210 ± 0.004 g/cm² vs. Cr = 0.206 ± 0.004 g/cm²; P > 0.49) and BMC (Pl = 0.407 ± 0.021 g vs. Cr = 0.385 ± 0.021 g; P > 0.46). Conclusion: In this study, using the experimental model of osteoporosis, Cr supplementation had no effect on bone mass.

Keywords: osteoporosis, creatine, bone mineral density.

INTRODUCTION

The spontaneously hypertensive rat (SHR) has been considered the arterial hypertension genetic model most similar to the primary hypertension observed in humans.1,2 There is evidence of a reduction in bone mineral density (BMD) in that model, resulting from changes in calcium metabolism, which cause an increase in bone resorption.3,3 While the bone mass of healthy Sprague Dawley rats peaks around the age of 30–36 weeks, SHRs stabilize their bone growth at the age of only 18 weeks. In addition, the peak bone mass in SHRs is approximately 40 mg/cm² lower than that in healthy rats.3 Thus, SHRs are considered an experimental model for the study of osteoporosis.1

The energy need of bone cells to survive, proliferate, differentiate, and synthesize extracellular matrix is known to be high.4 Evidence has shown that part of the energy required for those processes originates from creatine (Cr; α-methyl guanidine-acetic acid), which plays a central role in maintaining
ATP and ADP levels in several tissues, such as skeletal muscle, brain, testicles, cartilage, and bone (for a recent and comprehensive review, see Wallimann et al.9).

The hypothesis that Cr could play an important role in bone metabolism was first suggested based on the identification of creatine kinase isoforms (CK), enzyme responsible for the reversible reaction as follow: phosphocreatine + ADP + H+ ⇌ creatine + ATP in the bone.9,10 In addition, in vitro assays have shown that stimuli that can induce the development of bone mass (i.e., insulin growth factor-1 and parathyroid hormone) concomitantly increase CK activity, suggesting that the Cr/CK system is associated with the process of bone remodeling.11,12 In fact, a study has shown that incubating Cr in a culture medium with primary osteoblasts has stimulating effects on the differentiation, metabolic activity, and bone mineralization, elevating the phosphorylcreatine/Cr ratio and preserving the ultrastructure and mitochondrial function of osteoblasts.8

In vivo evidence13,14 has corroborated those findings. Supplementation with Cr can increase the BMD and cause beneficial biomechanical adaptations in the femur of healthy rats.15 In humans, there is preliminary evidence that the Cr supplementation can prevent bone mass loss in patients with Duchenne dystrophy14 and in the elderly undergoing physical training.16 However, the role of the dietary Cr supplementation remains little explored. Aiming at increasing the understanding about the effects of Cr on possible bone mass loss conditions, the present study assessed the effect of that nutrient on the BMD and bone mineral content (BMC) of SHRs.

MATERIALS AND METHODS

Sample

The sample comprised 16 8-month-old male SHRs, which were maintained at the animal facility of the Laboratory of Nutrition and Metabolism Applied to Motor Activity (Escola de Educação Física e Esporte of the Universidade de São Paulo – EEFE/USP) in plastic cages (three to four animals per cage), at room temperature of 22.0 °C–24.0 °C and 12-hour cycle (inverted light and dark). The rats were fed a normal protein diet (12% of protein) and water ad libitum.

Experimental design

The animals were randomized into two experimental groups matched by body weight, as follows: 1) Pl group: SHRs treated with placebo (n = 8); and 2) Cr group: SHRs treated with Cr (n = 8). After nine weeks of intervention the animals were sacrificed by decapitation. The bone specimens [right femur and lumbar spine L1–L4] were removed by use of surgical tools, immersed in saline solution, and frozen at −80 °C for later BMD analysis.

The procedures were approved by the Ethics Committee in Research of the EEFE/USP (protocol 2011/10).

Creatine supplementation

The Cr group received, through gavage, Cr supplement daily (Ethika, Ribeirão Preto, SP, Brazil) for nine weeks. Creatine powder was diluted in water (room temperature) at the proportion of 200 g for each liter of water, and the dosage used was 5 g/kg weight/day.13 The animals were weighted daily for the required corrections. The Pl group received distilled water through gavage to simulate the stress imposed to the Cr group.

Bone densitometry

Bone densitometry (Dual Energy X-Ray Absorptiometry; DXA) was used to assess the BMD and BMC of the spine (L1–L4) and total femur (total femur length, including diaphysis and epiphyses). The device Discovery-A SN: 80999 Hologic (Bedford, MA, USA) in the high resolution mode was used, with the aid of the small animal software, provided by the same manufacturer. The accuracy of the DXA for assessing BMD was previously analyzed by measuring the coefficient of variation, expressed as a percentage of the mean.17,18 The coefficient of variation was 1.9% for the spine and 0.6% for the total femur. Together, those data indicate high accuracy of measures.

Statistical analysis

Data were expressed as mean ± standard deviation. The non-paired t test was used to compare BMD and BMC of the groups and two-way ANOVA to assess body weight every week. The significance level adopted to reject the null hypothesis was P < 0.05.

RESULTS

Only one rat (Pl) died during follow-up. The body weight did not significantly differ between both groups during the study (P = 0.48; Figure 1).

No significant difference was observed between the groups regarding either the spine or the total femur measures as follows: spine – BMD (Pl = 0.249 ± 0.003 g/cm² vs. Cr = 0.249 ± 0.004 g/cm²; P = 0.95; Figure 2A), and BMC (Pl = 0.509 ± 0.150 g vs. Cr = 0.509 ± 0.017 g; P > 0.99;
DISCUSSION

This study aimed at assessing the influence of Cr supplementation in the bone mass of SHRs, a well-described experimental model to study low bone mass.\textsuperscript{3–5} Our results are not in accordance with those of Antolic et al.,\textsuperscript{15} who have reported beneficial effects of Cr supplementation on the bone mass of Sprague Dawley rats. Some methodological differences can explain the contradictory results. Antolic et al.\textsuperscript{15} have reported benefits with Cr supplementation to growing rats, while the present study used adult rats. The process of bone growth and development is characterized by high bone turnover, a period more susceptible to environmental influences on bone mass.\textsuperscript{19} The gains with Cr supplementation might have been intensified in that phase. In addition, it is worth emphasizing that the SHR model is known to have high bone resorption, and, thus, low bone mass. On the other hand, the Sprague Dawley model studied by Antolic et al.\textsuperscript{15} has no change in bone metabolism. Based on the differences of the experimental models, one can speculate that Cr supplementation might be more effective in potentiating bone mass gain in healthy growing rats than in attenuating bone mass loss in rats undergoing bone mass loss.
This study has some limitations. The tissue capture of Cr, to guarantee the success of supplementation, was not assessed. However, the dosage used in this study (5 g/kg weight/day) has been considered high in the literature\textsuperscript{20,21} and effective to increase the musculoskeletal content of Cr in Wistar rats.\textsuperscript{22} Future studies have to assess whether the Cr supplementation can also increase Cr concentrations in bone tissue. Finally, it is worth emphasizing that our study assessed only male rats. Knowing that gender is a factor that influences directly the bone mass response,\textsuperscript{22} and, considering the impossibility of generalizing those data for both genders, further studies should also assess the therapeutic potential of Cr in bone mass remodeling in females.

Despite the evidence that Cr supplementation can promote important therapeutic effects, such as bone mass increase,\textsuperscript{23} our findings indicate that SHRs supplemented with Cr do not experience such gains. Considering the evident difficulty in carrying out large longitudinal clinical assays, other models characterized by bone mass loss (for example, polycystic rats or rats treated with corticoids) should be investigated to more deeply assess the therapeutic potential of Cr on the preservation of BMD in low bone mass conditions. However, it is worth emphasizing that the Cr metabolism seems to differ substantially between species, and, thus, studies in humans should be conducted to confirm all preclinical findings.
de Cr poderiam ter sido acentuados nessa fase. Além disso, é importante ressaltar que o modelo SHR apresenta salientemente alta reabsorção óssea e, por conseguinte, baixa massa óssea. Em contrapartida, o modelo Sprague Dawley estudado por Antolic et al.\textsuperscript{15} não apresenta alterações no metabolismo ósseo. Com base nas diferenças dos modelos experimentais, é possível especular que a suplementação de Cr seja mais eficaz em potencializar o ganho de massa óssea em ratos saudáveis em fase de crescimento que atenuar a perda de massa óssea em ratos em processo de perda de massa óssea.

Este trabalho apresenta algumas limitações. Em primeiro lugar, não avaliamos a captação tissular de Cr a fim de garantir o sucesso da suplementação. Contudo, a dosagem empregada neste estudo (5 g/kg peso/dia) tem sido considerada alta na literatura\textsuperscript{20,21} e efetiva em aumentar o conteúdo musculoesquelético de Cr em ratos Wistar.\textsuperscript{21} Estudos futuros precisam avaliar se a suplementação do Cr é capaz de elevar as concentrações desse substrato também no tecido ósseo. Por fim, é importante ressaltar que apenas ratos machos foram avaliados em nosso estudo. Sabendo que o gênero é um fator que influencia diretamente na resposta da massa óssea\textsuperscript{22} e, portanto, dada a impossibilidade de generalização desses dados para ambos os gêneros, novos estudos devem também avaliar o potencial terapêutico do Cr no remodelamento da massa óssea em fêmeas.

Embora existam evidências de que a suplementação de Cr poderia promover importantes efeitos terapêuticos, incluindo aumento de massa óssea,\textsuperscript{23} nossos achados indicam que ratos SHR suplementados com Cr não apresentam tais ganhos. Tendo em vista a evidente dificuldade em se realizar grandes ensaios clínicos longitudinais, outros modelos que se caracterizam por perda de massa óssea (p. ex., ratos policísticos ou tratados com corticoides) devem ser investigados, a fim de se avaliar com maior profundidade o potencial terapêutico do Cr sobre a preservação da DMO em condições de baixa massa óssea. Contudo, cabe ressaltar que o metabolismo do Cr parece diferir substancialmente entre espécies, razão pela qual estudo em humanos deve ser conduzido com o intuito de comprovar todos os achados pré-clínicos.

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Description of a new method of ovariectomy in female rats
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ABSTRACT
Rats are currently the most used laboratory animals to investigate osteoporosis. We report an efficient method of ovariectomy and compared this method with the two other operative methods of ovariectomy (i.e., midline dorsal skin incision and double dorsolateral approach, which are used commonly for inducing experimental osteoporosis. Female Wistar rats, 12 weeks old, were divided into three groups. Ovariectomy was preceded by a single midline dorsal skin incision, 3 cm long, in the group A; double dorsolateral incisions, approximately 1 cm long, in the group B; and a single ventral transverse incision of 0.4–0.6 cm at the middle abdominal region in the group C. Animals in groups A, B, and C had a mean weight of 258.12 ± 0.54 g, 255.78 ± 0.42 g, and 254.55 ± 1.69 g, respectively. There were significant differences in the duration (in minutes) of surgery in the groups B (9.65 ± 0.86) and C (7.55 ± 0.11, P < 0.001) when compared to the group A (15.52 ± 0.30) and in the group B (P < 0.01) when compared to the group C. Wound healing time (in days) for groups B (9.22 ± 0.67) and C (8.01 ± 0.93) was significantly shorter than that for group A (11.58 ± 1.2, P < 0.001), with the wound healing time for group C being slightly shorter than that for group B. The surgery, as conducted in the group C, was technically easier, less time consuming and showed less wound healing duration.

Keywords: ovariectomy, postmenopausal osteoporosis, animal models.

INTRODUCTION
The understanding of postmenopausal osteoporosis is hindered by the difficulty of studying a disease that is restricted to humans. Therefore, the use of an animal model of postmenopausal osteoporosis provides more uniform experimental material and allows for assessment of potential therapies for it.1 For the study of postmenopausal osteoporosis, the use of an animal model will reduce the problems which are associated with studying the disease in humans, such as time and variability in the behavior of test subjects. Even clinical studies are highly expensive and require longer durations, which is one more reason that explains why animal models play an important role in understanding the osteoporosis research. In the United States, for the preclinical evaluation of new drugs, the Food and Drug Administration (FDA) requires data from animal models.2

The most commonly used animals to investigate osteoporosis are rats because they are inexpensive, easy to house, grow rapidly, widely available and the general public is accustomed to the role of rodents for use in research.3 Ovariectomy in female rats can be performed in different ways and the selection of the operative method for ovariectomy is very important, especially when the number of animals is very high and the duration of the experiment is short. There are mainly two types of incision used for doing ovariectomy in female rats: single midline dorsal skin incision4 and double dorsolateral incisions.5

In this study we focused on creating a minimally invasive method of ovariectomy which can be useful to veterinarians in private practice or in research facilities, as a model for inducing experimental postmenopausal osteoporosis in female rats. We also compared our novel and efficient method of ovariectomy with other reported methods for this surgery in terms of duration.
of the operative procedure, degree of difficulty of the operative technique and access to gonads and duration of wound healing.

MATERIALS AND METHODS

Animals

Twenty-four healthy in-house laboratory-bred Wistar rats with 12 weeks age were included for the study. The animals were maintained under controlled temperature at 25 °C ± 2 ºC with 12-hour light/dark cycle. They were fed standard laboratory diet (Amrut rat and mice feed, Pranav Agro Industries Ltd., Sangli, India) and water ad libitum. Before conducting the experiment, ethical clearance was obtained from the Institutional Animal Ethics Committee (IAEC) of Al-Ameen College of Pharmacy, Bangalore.

Surgical procedure: ovariectomy

Prior to the surgery the weight of the animals was measured by a digital weighing machine. Female Wistar rats were divided into three groups consisting of eight animals each. The animals were anesthetized with a combination of ketamine (80 mg/kg) and xylazine (10 mg/kg), intraperitoneally (Neon Pharma, Mumbai and Indian Immunologicals Ltd., Hyderabad, respectively).

In the group A, ovariectomy was preceded by a midline dor-sal skin incision, 3-cm long, approximately halfway between the middle of the back and the base of the tail according to the method described by Lasota et al. In the group B, ovariectomy was made by two dorsolateral incisions; one small incision (1.5 cm) was made through the skin and the muscle wall on each side of the backbone, in the dorsal aspect according to the method described by Park et al. In the group C, operation was made after placing an anesthetized animal on its dorsal surface. The fur on the rat abdomen was completely removed with depilatory cream (Veet, Reckitt Benckiser, India).

The area of surgery was cleaned with ethanol (Merck, India). A small transverse peritoneal incision of 0.4–0.6 cm was made with surgical scalpel blade no. 11 on the middle part of the abdo-men slightly towards right, just near to the second right nipple of the rat, as shown in Figure 1. After peritoneal cavity was accessed, the adipose tissue was pulled away until the right uterine tube and the ovary surrounded by a variable amount of fat were identified. The ovary and associated fat were easily located and exteriorized by gentle retraction. The procedure was repeated for the left ovary through the same incision, as shown in Figure 2. The periovarian fat with the ovary was pulled away from the incision site gently to prevent detachment of a small piece of ovary, which may fall into the abdominal cavity where it may be reimplanted and carry on its normal function. After identifying the ovary and uterine horn, a braided silk suture (Ethicon mersilk sutures-3/0, Johnson & Johnson Ltd., India) was performed around the area of the distal uterine horns, that was sectioned thereafter, and the ovaries were removed, as shown in Figure 3.

Figure 1
Transverse incision made on the middle part of abdomen slightly towards the right with a surgical scalpel blade. The transverse abdominal muscle is exposed after skin incision.

Figure 2
After the muscle dissection, the peritoneal space and adipose tissue surrounding ovary are exposed. Thick black circles show the ovary surrounded by adipose tissue.

Figure 3
Ligation at the distal uterine horn in order to completely remove the ovary, one at a time. The ovary surrounded by fat is completely removed (thick black circle).
The uterine horn was returned to the peritoneal cavity after the removal of ovaries. The wound was closed in two layers (muscle and skin) using sterile sutures. The peritoneum and the muscle layers were sutured with one absorbable suture (Ethicon chromic sutures-3/0, Johnson & Johnson Ltd., India) and the skin was sutured with one non-absorbable suture (Ethicon mersilk sutures-3/0, Johnson & Johnson Ltd., India). Povidone iodine was applied on the area to disinfect the skin after suturing. High degree of aseptic procedure was maintained throughout the operation.

After surgery, the rats were housed individually in polyurethane boxes for a period of one week to allow recovery and then re-grouped in their home cages. There was also some concern that the ventral approach in rodents means that the wound was in an almost constant direct contact with the paddy husk bedding, which may result in more frequent wound breakdowns. In order to prevent this, the animals after surgery were housed individually in polyurethane boxes provided with clean and dry bedding sets made of 100% sterilized cotton fabric for extra comfort and warmth for a period of one week in order to avoid hypothermia and to prevent possible contamination.

Evaluation of wound healing
To evaluate wound healing, duration of healing, absolute and normalized length area of the wound were used. The maximum length area was measured on the second day after surgery. Thereafter, this measurement was carried out every two days until full healing occurred. The healing percentage or the normalized values were calculated by dividing the maximum length of the wounds by that measured on the second day after surgery. The duration of wound healing was the time taken for full contraction of the wound. Wound healing percentage was calculated using the equation 
\[ \frac{(L_2-L_1)}{L_2} \times 100 \], where \( L_2 \) and \( L_1 \) are the maximum wound lengths on the second and any other day, respectively.

Statistical analysis
Results are given as mean ± SEM. One-way Analysis of Variance (ANOVA) followed by Bonferroni’s test did the comparisons between different groups. In all cases, a probability error of less than 0.05 was selected as the criterion for statistical significance.

RESULTS
Mortality rate
There was no procedure-related death reported in the group B. However, two animals from group A and one animal from group B died within two days after surgery. Any procedure that causes

Body weight
Before operation, there was no significant difference in the rat’s body weight between groups. Table 1 summarizes the effects of ovariectomy on body weight in all groups. There was no significant difference in the body weight of animals after surgery in all three groups.

Surgery duration
The surgery duration in group C (7.55 ± 0.11 min) was found to be significantly less than in groups A (15.52 ± 0.30 min, \( P < 0.001 \)) and B (9.65 ± 0.86 min, \( P < 0.01 \)), as shown in Table 1. Also in group B, the surgery duration was significantly lower when compared with group A (\( P < 0.001 \)).

Wound healing time
The wound healing time of groups B (9.22 ± 0.67 days) and C (8.01 ± 0.93 days) was significantly shorter than those of group A (11.58 ± 1.2 days, \( P < 0.001 \)), as shown in Table 1.

Table 1
Comparison of different surgical procedures of ovariectomy in rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Incision</th>
<th>Body weight (g)</th>
<th>Surgery duration (min)</th>
<th>Wound healing time (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A (n = 8)</td>
<td>Single midline dorsal, 3 cm long</td>
<td>258.12 ± 0.54</td>
<td>15.52 ± 0.30</td>
<td>11.58 ± 1.2</td>
</tr>
<tr>
<td>Group B (n = 8)</td>
<td>Two dorsolateral, each 1.5 cm long</td>
<td>255.78 ± 0.42</td>
<td>9.65 ± 0.86*</td>
<td>9.22 ± 0.67*</td>
</tr>
<tr>
<td>Group C (n = 8)</td>
<td>Abdominal transverse, 0.4–0.6 cm long</td>
<td>254.55 ± 1.69</td>
<td>7.55 ± 0.11**</td>
<td>8.01 ± 0.93*</td>
</tr>
</tbody>
</table>

Data are shown as mean ± SEM (n = 8), evaluated by Bonferroni’s test. All values are mean ± SEM; n = 8. *Significantly statistical comparison with group A (\( P < 0.001 \)); **Significantly statistical comparison with group B (\( P < 0.01 \)).
However, we observed no significant difference in the wound healing time of group C when compared with group B. The distributions of wound length and healing percentage per day showed significant variation between groups A and C. The variation of the wound length was different among all three groups (Figure 4).

DISCUSSION

Rodents are the most commonly used animal model for osteoporosis studies, because after ovariectomy female rat skeleton is more sensitive to the loss of ovarian hormones and exhibit most of the characteristics of human postmenopausal osteoporosis. Therefore, for the preliminary evaluation of a new pharmacological agent which might be effective in postmenopausal osteoporosis, rodents are generally the species of first choice, followed by verification in other species before undertaking clinical trials in human patients. However, the procedure for creating an efficient method of ovariectomy in female rats has not been reported in the published literature so far, to the best of our knowledge. Thus, in this article we describe a novel approach for creating a minimal invasive ovariectomy. We also compared our method with two other methods of ovariectomy which had been reported earlier (i.e., single midline dorsal skin incision and double dorsolateral approach).

Before making an abdominal incision, the surgeon needs to consider multiple factors such as the area that needs to be exposed, operative exposure, simplicity, and the need for quick entry into the abdominal cavity. The most important factor is an adequate exposure to the operative field. Complications during surgery can occur because of inadequate exposure, which is often due to the unwillingness of the surgeon to extend the incision. In this study, the duration of surgery in group C (ventral transverse incision, 0.4–0.6 cm long) was found to be significantly less than in groups A (single midline dorsal skin incision, 3 cm long) and B (double dorsolateral incisions, each 1.5 cm long).

A careful selection of the incision site and the proper closure of the wound after an abdominal incision are very important for the surgery success. The type of the incision may however have its influence on the occurrence of postoperative wound complications. The small transverse incision at the abdominal region is based on better anatomical and physiological principles. Reported advantages of transverse incisions for abdominal surgery include better cosmetic results, less pain, and low incidence of hernia formation.

The wound healing time of group C was significantly shorter than those of group A. No significant difference in the wound healing time of group C was observed when compared with group B, although the healing time of wounds in group C was lower when compared to group B. The results reported in this paper suggest the need of a precise muscle incision during an ovariectomy, which results in a quick location of the ovary in the female rats. Large muscle incisions or double dorsolateral incisions may lead to excess bleeding, requiring the use of more sutures.

CONCLUSION

By comparison of these different types of incisions for the ovariectomy in rats, it is suggested that the use of a ventral abdominal transverse incision rather than a single midline dorsal skin incision, or a double dorsolateral incision may influence parameters like duration of surgery, recovery, and complication rates. Compared to groups A and B, the operation conducted in group C was technically easier, less time consuming, and with faster wound healing.

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única na linha média, ou incisões dorsolaterais duplas, possa influenciar parâmetros tais como duração da cirurgia e taxas de recuperação e complicação. A operação realizada no grupo C, quando comparada àquelas realizadas nos grupos A e B, mostrou-se tecnicamente mais fácil, consumiu menos tempo e apresentou cicatrização de ferida mais rápida.

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REFERENCES