Sociedade Portuguesa de Reumatologia

Acta Reumatológica Portuguesa

Publicação Trimestral • ISSN: 0303-464X • 10 €

Vol 33 • Nº 1
Janeiro/Março 2008

Medfarma edições

Acta Reumatológica Portuguesa

Administração, Direcção Comercial e Serviços de Publicidade
Medfarma - Edições Médicas, Lda
Alameda António Sérgio 22, 4º B
Edif. Amadeo de Souza-Cardoso
1495-132 Algés
Tel: 214 121 142
Fax: 214 121 146

Redacção
Sociedade Portuguesa de Reumatologia
Rua D. Estefânia 177, 1º D
1000-154 Lisboa

Depósito Legal: 86.955/95

Tiragem: 7.500 exemplares

Registo
Isenta de inscrição no I.C.S. nos termos da alínea a) do n.º 1 do artigo 12.º do Decreto Regulamentar n.º 8/99, de 9 de Junho.

Preço: 10 €

Impressão e Acabamento
Óptima Tipográfica, Lda.
Casais da Serra • 2665-305 Milharado

Produção Gráfica
Rita Correia

Periodicidade
Publicação Trimestral

Esta edição contém um SUPLEMENTO que não pode ser distribuído separadamente.

Revista referenciada no Index Medicus, Medline, Pubmed desde Janeiro 2006.
Journal referred in Index Medicus, Medline, Pubmed since January 2006.

O papel utilizado nesta publicação cumpre os requisitos da ANSI/NISO Z39.48-1992 (Permanence of Paper).
The paper used in this publication meets the requirements of ANSI/NISO Z39.48-1992 (Permanence of Paper).

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ACTA REUMATOLÓGICA PORTUGUESA

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ATEROSCLEROSE, INFLAMAÇÃO E DOENÇAS REUMÁTICAS

Maria José Santos*

O tratamento das doenças reumáticas inflamatórias sofreu avanços significativos nas últimas décadas. Graças ao progresso no conhecimento dos mecanismos fisiopatológicos das doenças e ao surgimento de novos fármacos, a prevenção de lesões estruturais irreversíveis e mesmo a remissão clínica são hoje em dia objectivos terapêuticos passíveis de serem alcançados. Numa percentagem elevada de doentes consegue-se um controlo eficaz da inflamação, sendo possível, em grande medida, evitar as deformações articulares e possibilitar uma vida quase normal e com melhor qualidade.

Contudo, as doenças reumáticas podem ter repercussões muito para além da articulação e, apesar de todos os avanços terapêuticos, a esperança de vida dos doentes com doenças reumáticas sistémicas está diminuída, em parte devido a um excesso de mortes por patologia cardiovascular (CV).

A elevada prevalência de aterosclerose subclínica e de eventos CV em idades jovens, sobejamente documentada no Lúpus Eritematoso Sistémico (LES), mas também na Artrite Reumatóide (AR), Artrite Psoriática ou Espondilite Anquilosante é um desafio para todos os clínicos que lidam com estas patologias. Nos doentes com AR o risco de eventos CV é 2-3 vezes mais elevado do que na população em geral e nos doentes com LES esse risco é 5-6 vezes superior. Esta diferença é ainda mais notória nas mulheres antes da menopausa.[1-3]

Actualmente a aterosclerose é reconhecida como uma doença multifactorial para a qual contribuem determinantes genéticos, factores de risco CV tradicionais e mediadores da inflamação, mas a interacção e o contributo relativo dos vários factores não está totalmente esclarecido.

Aterosclerose uma doença inflamatória

A inflamação é uma constante em todas as etapas do processo aterosclerótico desde a activação e disfunção do endotélio, à formação e ruptura da placa aterosclerótica, responsável pela maioria dos eventos cardiovasculares. Nas fases mais precoces deste processo parece estar a disfunção do endotélio e infiltração da íntima por linfócitos T activados (CD4+, HLA-DR+ e IL-2R+) e monócitos/macrófagos, responsáveis pela formação das chamadas estrias gordas, presentes mesmo em idades muito jovens *(lesão inicial ou lesão mínima)*. Há produção local de vários mediadores da inflamação tais como a interleucina (IL)-1, factor de necrose tumoral alfa (TNF-alfa), linfotoxina alfa (LTA), IL-2, IL-6, IL-8 e interferão gama, que podem perpetuar a reacção inflamatória no interior da lesão aterosclerótica inicial. A inflamação persistente promove a migração e proliferação das células musculares lisas, a remodelação da parede arterial e a formação de *lesões intermédias* (pré-ateroma).[4] A disfunção endotelial e a inflamação crónica da parede arterial contribuem para alterações hemorreológicas e favorecem a agregação eritrocitária. Com a continuação deste processo pode ocorrer necrose focal. A reparação destas lesões com tecido fibrótico que cobre um núcleo contendo lípidos e tecido necrótico, estreita o lúmen dos vasos e altera o fluxo sanguíneo *(lesão aterosclerótica avançada)*. As lesões avançadas tendem a desenvolver fissuras à superfície, hematomas e trombose. O hematoma e o trombo reduzem subitamente o lúmen arterial e são frequentemente responsáveis pela morbilidade e mortalidade associadas à aterosclerose. As calcificações são habituais nas lesões ateroscleróticas evoluídas, principalmente em doentes mais idosos.

Contributo da inflamação para a aterosclerose

Os mediadores da inflamação podem contribuir para o processo aterosclerótico, ainda que a relação entre estes mediadores e o processo aterogénico não esteja cabalmente esclarecida.

Por um lado, a existência de um processo inflamatório sistémico, documentado pela elevação dos níveis séricos de proteína C reactiva, IL-1, IL-6 ou

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factor de necrose tumoral alfa, é um marcador de risco independente para a ocorrência de eventos CV na população em geral.[5,6] Admite-se que nos doentes reumáticos o processo inflamatório inerente à própria doença contribui, *per se*, para a aterosclerose precoce. O TNF-alfa é uma das citocinas proinflamatórias crucial na patofisiologia de algumas doenças reumáticas, que se destaca pelo seu contributo para a aterogénese. Esta citocina aumenta a expressão de moléculas de adesão a nível do endotélio, promove o recrutamento de células inflamatórias para a parede arterial e é fundamental no processo inflamatório dentro da parede arterial.[7]

Por outro lado, a inflamação persistente interfere e pode modificar alguns factores de risco CV tradicionais, estando documentada a associação entre o processo inflamatório e um perfil lipídico mais aterogénico e também um agravamento da insulinoresistência. Mais uma vez o TNF-alfa, citocina pró-inflamatória por excelência, é responsável pelo aumento dos níveis de triglicéridos, diminuição do colesterol total e do C-HDL e pode induzir alterações estruturais no C-LDL, tornando estas partículas mais aterogénicas.[7]

Alguns marcadores genéticos podem constituir um elo entre a inflamação, a ocorrência de doença reumática e a aterosclerose. Disso são exemplo polimorfismos do promotor do gene do TNF-alfa que predispõem para doença coronária aterosclerótica e são também um marcador de prog- nóstico da AR ou polimorfismos do gene da LTA que se associam a aumento dos níveis séricos de proteína C reactiva e simultaneamente a maior risco de aterosclerose e eventos cardiovasculares.[8,9]

Factores de risco tradicionais e menos tradicionais

Embora os factores de risco CV tradicionais, tais como a idade, o sexo masculino, a dislipidemia, a diabetes, o tabagismo ou a história familiar de doença cardiovascular prematura, por si só, não explicarem a prevalência aumentada de aterosclerose nas doenças reumáticas inflamatórias, a sua presença contribui para o aumento do risco. À semelhança do que sucede na população geral, também nos doentes reumáticos os níveis elevados de C-LDL, radicais livres causados pelo fumo do tabaco, hipertensão arterial, diabetes, predisposição genética, hiperhomocisteinemia e algumas infecções (por ex. herpes vírus ou *chlamydia)* são factores que podem causar ou favorecer a disfunção endotelial. Para além disso, a acumulação de lípidos na íntima fica facilitada quando os níveis de lipoproteínas no plasma e a pressão sanguínea excedem os valores normais.

A terapêutica da própria doença reumática, nomeadamente a corticoterapia e a presença de lesão renal são outros factores a ter em consideração no processo aterosclerótico.

Mais recentemente, aumentos da homocisteína e da dimetilarginina assimétrica (ADMA) foram associados a um aumento do risco de doença aterosclerótica e de fenómenos trombóticos.[10] Este facto pode ter particular relevância para o reumatologista, pois fármacos tão frequentemente utilizados como são o metotrexato ou a sulfassalazina elevam os níveis séricos de homocisteína.[11]

Na avaliação do risco cardiovascular são por vezes usados índices compostos *(Score de Framingham)* ou avaliada a presença da Síndrome Metabólica que, na população em geral, têm um melhor valor preditivo do que cada factor de risco CV individualmente.[12,13] O valor destes índices é questionável nas doenças reumáticas, pois tudo indica que a equação de Framingham subestima o real risco CV destes doentes.[14] Numa avaliação que realizámos em doentes com LES, o risco CV a 10 anos avaliado pela equação de Framingham foi inferior ao da população controle de idênticas características demográficas, mas em contrapartida a Síndrome Metabólica foi significativamente mais prevalente na população com LES (dados não publicados).

O interesse de vários autores pela problemática da aterosclerose nas doenças reumáticas está patente neste número da Acta Reumatológica Portuguesa onde é publicada uma revisão detalhada dos mecanismos implicados na patogenia da aterosclerose nos doentes com AR e da utilização do *eco-doppler* carotídeo como método não invasivo de diagnóstico precoce de lesões ateroscleróticas nestes pacientes.[15] Num outro artigo Terreri e col. alertam para concentrações persistentemente elevadas de homocisteína, reconhecido factor de risco CV, em 5 (27,6%) das 18 crianças e adolescentes com LES Juvenil avaliadas.[16]

É importante estar atento às possíveis complicações tardias das doenças reumáticas inflamatórias, saber reconhecê-las atempadamente, mas sobretudo intervir de forma eficaz na sua prevenção. Falta promover estudos que permitam em defini-

tivo responder à dúvida: qual ou quais as intervenções efectivas na prevenção da aterosclerose prematura associada às doenças reumáticas inflamatórias?

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MANAGEMENT OF CARDIOVASCULAR RISK FACTORS IN PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS

Anisur Rahman*

Cardiovascular problems including coronary artery disease and strokes are a major cause of death in patients with SLE, particularly during the later stages of the disease. There is abundant evidence that the risk of cardiovascular disease (CVD) is increased in patients with SLE compared to age and sex matched controls.[1-3] Although cardiovascular risk is also raised in other rheumatological diseases such as rheumatoid arthritis, the increase is particularly striking in SLE because most patients with that disease are young or middle-aged women who would normally have a very low incidence of CVD. Manzi *et al* [1] showed that women aged 35-44 with SLE had a 50-fold higher incidence of coronary disease than age-matched controls from the Framingham cohort. Studies from groups in different countries have all confirmed this increased risk, though not with such high relative risk values (reviewed in [4]).

The incidence of subclinical arterial disease is also increased in patients with SLE. This has been shown by post-mortem studies[5] showing that 52% of patients with SLE had atherosclerosis and by imaging studies. Roman *et al*[6] used ultrasound to show that carotid artery narrowing (measured as intima/media thickness (IMT)) occurs more frequently in patients with SLE than in age/sex matched control subjects while Asanuma *et al*[7] used electron beam tomography to show that coronary artery calcification occurs more frequently in patients with SLE than in controls. However, neither carotid artery IMT nor coronary calcification has been shown to be an accurate predictor of CVD events in patients with SLE.

Why do patients with SLE have increased risk of atherosclerosis and CVD? One possibility is that this risk arises from the same factors that are known to be the major contributors to CVD risk in the general population, including smoking, hypertension, high cholesterol and diabetes. Information about the presence of these factors in an individual can be used to calculate his or her predicted risk of developing coronary disease or stroke in the next ten years, using equations derived from the analysis of factors that predicted CVD events in the Framingham cohort.[8] These predicted risk values have been used widely in deciding how to manage cardiovascular risk in the community – for example by deciding which patients would benefit most from reduction of cholesterol.[9] In patients with SLE, however, it is clear that these risk calculations seriously underestimate the true risk of developing CVD. Esdaile *et al* carried out a retrospective study of 263 patients in two Canadian centres and found that even after allowing statistically for the effects of all orthodox risk factors, patients with SLE still had a 7.9-fold increase in the risk of stroke and a 10.1-fold increase in risk of non-fatal myocardial infarction.[2] Our group calculated predicted CVD risk for 202 patients with SLE in comparison with hypothetical age/sex matched controls in whom management of all risk factors was optimized and found that this optimization would only lead to a significant change in predicted risk in patients under 40.[3] In a group of 47 patients with SLE followed from 1992 to 2002, 8.5% developed coronary disease and 10.6 suffered stroke. This was a far higher incidence of CVD than would have been predicted by risk calculations on these patients in 1992 and the patients who suffered events were not those who had a high predicted risk of CVD in 1992.[3]

It is clear, therefore, that the standard risk factors used in the Framingham risk equations account for only part of the increased risk of CVD in patients with SLE. Case-control studies have suggested that a number of other factors contribute, including anti-phospholipid antibodies (aPL), raised triglyceride levels, and not being treated with hydroxychloroquine.[4,10,11] It has also been proposed that inflammation due to high disease activity[4,6] or high levels of homocysteine[12] might play a role in causing increased risk of CVD in patients with SLE. Some of these potential risk factors could be influenced by changing our management of patients with SLE, for example by prescribing folic acid to

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reduce homocysteine[12] or by adopting a lower threshold for anticoagulation in patients with aPL but no history of thrombosis. However, there is no evidence base to show that these measures would reduce cardiovascular risk in these patients. It is important to note that the absolute risk of CVD events in an individual patient with SLE is usually low, even when the relative risk compared to other people of the same age is high. This is an argument against prescribing drugs to reduce this relative risk without persuasive evidence of efficacy.

There is a strong evidence base for use of interventions affecting smoking, hypertension and lipid levels to reduce cardiovascular risk in the general population. Large, well-designed clinical studies showing the efficacy of such measures have been used to formulate guidelines for use of these interventions in primary prevention of CVD.[9] These guidelines, however, are not easily applicable to patients with SLE. Many of the trials used to derive them contained few or no young women and are therefore of limited relevance to patients with lupus. Furthermore, the guidelines generally depend on the predicted risk of CVD, calculated using the Framingham algorithm.[8] This method underestimates risk in patients with SLE, and gives calculated values for these patients far lower than would normally trigger use of statins under primary prevention guidelines. For example, the National Cholesterol Education Adult Treatment Panel III guidelines define high and moderate risk respectively as >20% and 10% to 20% predicted risk of coronary disease over the next ten years.[9] Most patients with lupus have far lower predicted risk values than this. In our cohort of 202 patients, the median 10-year risk values for coronary disease and stroke were 1.2% and 0.8% respectively.[3]

The fact that we cannot use these guidelines does not mean that we should not attempt to control reversible cardiovascular risk factors in patients with SLE. On the contrary, studies have shown that smoking, high cholesterol and hypertension are all common in cohorts of patients with lupus.[3,4] Most authors agree that it is important to address these potentially reversible risk factors in patients with a disease such as SLE that itself increases cardiovascular risk.[13,14] Wajed *et al*[14] argued that SLE, like diabetes mellitus, should be considered such a strong risk factor in its own right that all patients with the disease should be considered as being at high risk of CVD regardless of calculated risk values. This would imply instituting measures to cause the patient to stop smoking and to aim for target blood pressure and low density lipoprotein (LDL) cholesterol of 130/80 mmHg and 2.6mmol/l respectively.[14] This setting of standard targets applicable to all patients with SLE is attractive in its simplicity, but could lead to long term use of drugs such as statins in patients who do not really need to take them, because risk levels for the individual patient are not taken into account. However, in a recent questionnaire study[15] soliciting the opinions of lupus specialists from 32 units across Europe and North America, we found considerable support for the idea of assertive management of reversible risk factors without calculating predicted risk values in individual patients. All respondents to the questionnaire said they would encourage their patients with lupus to stop smoking, 55% would prescribe statins to any patient with lupus and high cholesterol and 74% would try to keep blood pressure between 120/80 and 140/80 mmHg.

In summary, patients with SLE have a high risk of CVD but only part of this is due to reversible orthodox risk factors. Calculation of risk scores using Framingham equations is misleading as it underestimates risk and does not identify the patients who are actually going to suffer an event. There is a general consensus that smoking, hypercholesterolaemia and hypertension should be treated in patients with SLE but at present there are no generally agreed guidelines about the target levels that we should aim for in treating these factors.

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13th International Conference for Behçet's Disease

Austria, Pörtschach/Klagenfurt
24-27 de Maio de 2008

TREGS AND RHEUMATOID ARTHRITIS

Amy E. Anderson, John D. Isaacs*

Abstract

Regulatory T cells (Tregs) are a subset of T cells which are involved in peripheral immune tolerance. Their role in autoimmune disease, which occurs through a breakdown of tolerance, is of particular interest in trying to ascertain the mechanism(s) of disease progression. It is hoped that by understanding the role of Tregs in autoimmunity a reliable therapy may be developed to aid in both the treatment and, potentially, cure of disease. This review will focus on the naturally-occurring $CD4^+CD25^+$ regulatory T cell subset and their possible involvement in rheumatoid arthritis.

Keywords: Treg, $CD4^+CD25^+$, rheumatoid arthritis, autoimmunity, tolerance

Resumo

As células T reguladoras (Tregs) são um subtipo de linfócitos T envolvido na tolerância imunológica periférica. O seu papel nas doenças autoimunes, que surgem por um mecanismo de quebra de tolerância, reveste-se de particular interesse numa tentativa de esclarecer o(s) mecanismo(s) de progressão da doença. Espera-se que, compreendendo o papel das Tregs na autoimunidade, se possa desenvolver uma terapêutica fiável que ajude no tratamento e potencialmente na cura da doença.

Esta revisão foca o subtipo de células T reguladoras que ocorre naturalmente, as células T CD4+CD45+, e o seu possível envolvimento na artrite reumatóide.

Palavras-chave: Treg; CD4+CD25+; Artrite Reumatóide; Autoimunidade, Tolerância.

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Autoimmunity and tolerance

Autoimmune diseases, e.g. type 1 diabetes, and rheumatoid arthritis (RA), affect about 5% of the World's population. They arise due to a break down of immunological self-tolerance. Ordinarily there are a number of mechanisms in place which control self-reactive T cells. Clonal deletion (negative selection) is the primary mechanism and occurs in the thymus during the development of the immune system. This mechanism is referred to as central tolerance and involves the deletion of T cells expressing a TCR with a high avidity for autoantigen expressed on thymic APCs. However, this system is imperfect and some self-reactive T cells escape this elimination process, making their way into the periphery where they have the potential to cause devastating damage.

To regulate these potentially self-destructive T cells a number of mechanisms exist in the periphery. These mechanisms are broadly termed peripheral tolerance and include clonal anergy, peripheral deletion, immunological ignorance and a variety of regulatory T cells (Tregs). Clonal anergy occurs when T cells encounter processed antigen in the absence of co-stimulatory signals. Anergised T cells subsequently cannot become activated even in the presence of full activating signals and therefore cannot mount an immune response. Peripheral deletion contributes to the elimination of T cells with a high avidity for antigen and to the deletion of T cells when the immune response is no longer required. It occurs through activation-induced cell death, whereby T cells repeatedly stimulated by antigen are deleted by apoptosis. This mechanism limits hypersensitivity reactions to allergens and autoantigens. Immunological ignorance refers to the situation whereby self-reactive T cells, although capable of mounting an immune response to their autoantigen don't respond to, or "ignore", it. This can arise for two reasons. The first is that the autoantigen may be present in a too low concentration. All T cells have a threshold for receptor occupancy which is necessary to trigger a response. Very low

concentrations of antigen will simply not be sensed. The second reason involves the sequestering of antigens in locations which are not freely exposed to immunological surveillance, e.g. the eye or central nervous system. T cells will simply not have access to their autoantigen. The final and probably the major mechanism of peripheral tolerance is existence the regulatory T cell (Treg) subsets.

Naturally-occurring CD4+CD25+ Tregs

Development

The most studied subset of Tregs is the naturally--occurring $CD4^+CD25^+$ population, which make up 5-10% of $CD4^+$ T cells in peripheral blood. In humans, it is the 1-2% of cells which have high CD25 expression that have suppressive activity and as such some reports refer to $CD4^+CD25^+$ naturally-occurring Tregs as $CD4^+CD25^{bright}$ or $CD4^+CD25^{high}$ Tregs. $CD4^+CD25^+$ Tregs cells are thought to arise in the thymus during thymic development. In fact it has been shown that $CD4^+CD25^+$ Tregs express self--reactive TCR.[1] High avidity interactions between the TCR and expressed autoantigen promote $CD4^+CD25^+$ Treg selection.[2] Lower avidity interactions promote the development of $CD4^+CD25^-$ effector T cells, whereas even higher avidity interactions lead to clonal deletion, as mentioned above. Recently, Vukmanovic-Stejic *et al.* have suggested that during adulthood a proportion of the Treg population is generated from highly differentiated memory $CD4^+$ T cells.[3] This may explain why Tregs are maintained throughout life, despite a decrease in thymic output with age.

Properties and function

In vitro $CD4^+CD25^+$ Tregs have properties of anergic cells upon stimulation characterised by low proliferation and low IL-2 production. However, it has been demonstrated that these cells have a high rate of proliferation *in vivo*.[3] Sakaguchi *et al.*, were the first to define $CD4^+CD25^+$ Tregs.[4] They reported that the transfer of T cells depleted of the $CD4^+CD25^+$ subset into athymic mice caused spontaneous development of various T cell-mediated autoimmune diseases, suggesting that $CD4^+CD25^+$ Tregs are involved in the suppression of self-reactive effector T cells. *In vitro* assays have shown that $CD4^+CD25^+$ Tregs are capable of suppressing polyclonal $CD4^+CD25^-$ T cell proliferation and cytokine production, especially IL-2, in a dose-dependent manner.[5-7] $CD4^+CD25^+$ Tregs have also been shown to directly suppress monocytes and macrophages[8] and DCs[9], thereby affecting both innate and adaptive immune responses. Both $CD4^+CD25^+$ Treg anergy and their suppressive function can be overcome by the addition of exogenous IL-2 or anti-CD28 antibody.[5-7]

Mechanisms of suppression and phenotypic identification

Suppression by $CD4^+CD25^+$ Tregs is thought to be mediated by a cytokine-independent, cell contact–dependent mechanism that requires activation of the Treg via the TCR.[5,6] Although these cells require antigen-specific activation, they are generally able to suppress T cell responses through bystander suppression once activated. The exact factors which are involved in suppression have not been fully elucidated and the mechanism by which suppression is achieved is controversial. $CD4^+CD25^+$ Tregs have been found to express IL-10, IL-4[5,10], TNF-α[5,10]and TGF-β[10]mRNA, but these cytokines were not detectable in anti-CD3-stimulated $CD4^+CD25^+$ Treg culture supernatants.[5,6] Other studies have reported that $CD4^+CD25^+$ Tregs do produce IL-10[11-13], IL-4[12] and TGF-β.[13,14] However, there is evidence which suggests that these cytokines do not play a role in $CD4^+CD25^+$ Treg-mediated suppression as the addition of neutralising anti-IL-10, anti-IL-4 and anti-TGF- α antibodies to *in vitro* mixed cultures of $CD4^+CD25^-$ and $CD4^+CD25^+$ Tregs failed to reverse suppression.[5,6] It is widely agreed that $CD4^+CD25^+$ Tregs do not produce IL-2.

Cytotoxic T lymphocyte-associated protein 4

As the mechanism by which $CD4^+CD25^+$ Tregs exert their effect is thought to be cell-contact dependent the presence of certain surface markers may be important in both the function and phenotypic characterisation of these cells. $CD4^+CD25^+$ Tregs express an array of surface molecules; however, the majority of these are not limited to $CD4^+CD25^+$ Tregs. For example, CD25 and cytotoxic T lymphocyte-associated protein 4 (CTLA-4) are both constitutively expressed on $CD4^+CD25^+$ Tregs but are also up-regulated on $CD4^+$ T cells after activation. CTLA-4 binds to CD80/CD86 ligands on APC and transduces a negative signal that results in down-regulation of T cell activation. The role of CTLA-4 in $CD4^+CD25^+$ Treg suppression is controversial. Some studies suggest that CTLA-4 is required for

suppressor function[15,16], whereas others have reported that it is not.[5] Interestingly, there are studies which report a genetic linkage between CTLA-4 and various autoimmune diseases, such as type 1 diabetes[17,18] systemic lupus erythematosus (SLE)[19], and Graves' Disease.[19,20] Genetic studies investigating the link between CTLA-4 polymorphisms and RA show conflicting results. However, CTLA-4 polymorphisms may play a role in RA susceptibility in the Asian population, but not the European population.[21]

Tumour necrosis factor receptor family – GITR and OX40

$CD4^+CD25^+$ Tregs also express high levels of the glucocorticoid-induced tumour necrosis factor receptor family-related protein (GITR). The ligand for GITR, GITR ligand (GITR-L), is expressed on APC and ligation of GITR to its ligand results in a co-stimulatory effect, enhancing $CD4^+$ T cell proliferation. $CD4^+CD25^+$ Treg suppressor function has been shown to be abrogated by the addition of agonisitic anti-GITR antibodies.[22,23] However, it has been shown that GITR engagement on $CD4^+CD25^-$ effector T cells, not $CD4^+CD25^+$ Tregs is responsible for the abrogation of suppression.[24] It should be noted that, like CD25 and CTLA-4, GITR is not $CD4^+CD25^+$ Treg-specific and is up-regulated on $CD4^+$ T cells upon activation. Another member of the tumour necrosis factor receptor family with co-stimulatory properties, OX40 (CD134), has been implicated in murine Treg function.[25] However, like GITR this molecule is not $CD4^+CD25^+$ Treg-specific and is expressed on naïve T cells as well as being transiently expressed on activated T cells.

Lymphocyte activation gene-3

Lymphocyte activation gene-3 (LAG-3) is another surface molecule which has been proposed as a $CD4^+CD25^+$ Treg-specific marker and has been implicated in $CD4^+CD25^+$ Treg function.[26] LAG-3 is a CD4-related molecule that binds MHC class II. LAG-3 has been reported to be selectively up-regulated on $CD4^+CD25^+$ Tregs after activation and anti-LAG-3 antibodies inhibited suppression by $CD4^+CD25^+$ Tregs.[26] Interestingly, ectopic expression of LAG-3 on $CD4^+$ T cells depleted of $CD25^+$ T cells conferred regulatory activity to these cells.[26] However, it should be noted that the above study was carried out in a murine model and LAG-3 was detected not only in naturally-occurring $CD4^+CD25^+$ Tregs but also in an induced Treg subset. Further work needs to be carried out to ascertain whether LAG-3 expression is relevant in human Treg biology and which Tregs subsets express this marker.

Neuropilin-1

Neuropilin-1 (Nrp1) is a receptor which is involved in axon guidance, angiogenesis, cell survival, migration and invasion. It has been reported that Nrp1 is constitutively expressed on the surface of $CD4^+CD25^+$ Tregs independently of their activation status, whereas Nrp1 expression is down-regulated in naïve $CD4^+CD25^-$ T cells after activation.[27] Nrp1 is co-regulated with Foxp3 (see below) and $CD4^+Nrp1^{high}$ T cells are able to suppress $CD4^+CD25^-$ T cells. Therefore, Nrp1 may be considered a candidate surface marker of $CD4^+CD25^+$ Tregs.

Foxp3

Foxp3 is a member of the *forkhead box* family of transcription factors. Over the past few years work has demonstrated that Foxp3 is critical for the development and function of $CD4^+CD25^+$ Tregs and therefore this intracellular marker appears to be of great significance for both the identification and function of $CD4^+CD25^+$ Tregs. Brunkow *et al.* first identified Foxp3 as a gene which is mutated in the mouse strain, *scurfy*.[28] They reported that this mutation was responsible for the presence of the fatal autoimmune lymphoproliferative disease found in *scurfy* mice. A similar disease present in humans, called immune dysregulation, polyendocrinopathy, enteropathy X-linked syndrome (IPEX) has also been mapped to a number of different mutations in the human Foxp3 gene.[29,30] Further work revealed that Foxp3 was associated with $CD4^+CD25^+$ Tregs and was critical for both their development and function.[31-33]

In mice Foxp3 expression is limited to $CD4^+CD25^+$ Tregs and unlike CD25 and other suggested Treg markers is not induced in $CD4^+CD25^-$ effector cells upon activation.[31-33] However, Walker *et al.* demonstrated that in humans Foxp3 expression is induced in stimulated $CD4^+CD25^-$ effector cells.[34] Expression of Foxp3 in these activated cells correlated with suppressive function. In agreement with this, a recent study has reported that activation-induced Foxp3 expression in human T cells leads to acquisition of a regulatory phenotype: these cells are able to suppress *in vitro* proliferation of autologous $CD4^+CD25^-$ T cells.[35] In contrast to these observations, Wang *et al.* reported that Foxp3 is

transiently expressed in activated $CD4^+$ T cells.[36] They demonstrated that induced Foxp3 expression leads to hyporesponsiveness in these cells but does not lead to acquisition of a regulatory phenotype. Interestingly, overexpression and ectopic expression of Foxp3 in non-regulatory T cells renders them suppressive.[31-33] The suppressive function of these cells is independent of CD25 expression, indicating that, at least in mice, CD25 is not required for regulatory activity.

Foxp3 has been implicated in the transcriptional regulation of cytokine genes and cell surface molecules. Two recent studies have identified Foxp3 binding regions in a number of genes.[37,38] Another recent paper has shown that Foxp3 expression in non-regulatory T cells leads to repression of genes, such as *IL-2* and *IFN-γ*, and induction of genes, such as *CD25*, *GITR*, and *CTLA-4*.[39] Interestingly, TGF-β has been shown to induce Foxp3 in $CD4^+CD25^-$ T cells resulting in T cells with suppressive activity.[40-42] The latter study has shown that IL-2 is essential for this TGF-β-induced effect.

Therefore, Foxp3 is a master regulator in the development of Tregs and may programme the conversion of non-regulatory cells to Tregs possessing suppressive activity. However, Foxp3 may not be a specific marker for naturally-occurring $CD4^+CD25^+$ Tregs and caution should be used when interpreting human Foxp3 studies.

CD27 and CD127

Recently, two further surface molecules have been identified which could aid in the identification of $CD4^+CD25^+$ Tregs. The first was identified in T cells from the synovial fluid of juvenile idiopathic arthritis (JIA) patients.[43] It was found that CD27 could be used in conjunction with CD25 to identify $Foxp3^+$ Treg cells: $CD4^+CD25^+CD27^+$ expressing cells were found to express high amounts of Foxp3, did not produce IL-2, IFN-γ or TNF and suppressed T cell proliferation; whereas $CD4^+CD25^+CD27^-$ cells expressed low amounts of Foxp3, produced effector cytokines and did not suppress T cell proliferation. However, a more recent paper has found that CD27 may not be confined to Tregs and as such cannot be used to reliably identify naturally-occurring $CD4^+CD25^+$ Tregs.[44] The second recent marker to be identified is CD127 (IL-7 receptor).[45,46] It was found that CD127 expression is inversely correlated with Foxp3 and suppressive function in human $CD4^+$ Treg cells. CD127 could prove useful in the search for a biomarker for the identification of human Tregs if it is used in conjunction with other markers.

Type-1 T regulatory cells

Type-1 T regulatory (Tr1) cells are another distinct subset of regulatory T cells (reviewed by Roncarolo *et al.*, 2006)[47]. Tr1 cells are not produced in the thymus but are induced in the periphery by antigen stimulation via an IL-10-dependent process. It has been demonstrated that Tr1 cells can also be induced by immature and tolerogenic DCs.[48] Unlike naturally-occurring $CD4^+CD25^+$ Tregs, Tr1 cells do not express high levels of CD25 or Foxp3. They produce high levels of IL-10 and TGF-β, which are thought to play a role in their antigen-specific regulatory activity. Furthermore, it has been shown that Tr1 cells can modulate immune responses *in vivo* in autoimmunity, transplantation, and chronic inflammatory diseases.

T helper 3 cells

T helper 3 (Th3) cells are another subset of induced regulatory T cells which are important in mucosal immunity (reviewed by Faria and Weiner, 2006)[49]. Th3 cells are primarily induced after ingestion of foreign antigen via the oral route (oral tolerance). High levels of TGF-β in the gut help promote the differentiation of naïve T cells into Th3 cells. Like naturally-occurring $CD4^+CD25^+$ Tregs, they express CTLA-4 on their surface and CD25 and Foxp3 expression is up-regulated after restimulation. However, the main mechanism of suppression is through the production of TGF-β. Th3 cells are triggered in an antigen-specific manner, but they can suppress in an antigen-non-specific manner through bystander suppression. In addition, they have been shown to suppress systemic inflammatory autoimmune responses.

Other types of Tregs

Other cells which have regulatory functions include subpopulations of $CD4^+$ and $CD8^+$ T cells: γδ T cells have been implicated in tissue immunoregulation[50]; and $CD8^+CD28^-$ Tregs play a role in suppression of autoimmunity.[51] Natural killer T (NKT) cells also have suppressive activity.[52] They produ-

ce regulatory cytokines, including IL-10, TGF-β, and can affect the Th1/Th2 balance. B cells subsets have also been implicated in immunoregulation.[53]

The remainder of this review will focus on naturally-occurring $CD4^+CD25^+$ Tregs and their role in autoimmune disease, in particular RA.

Evidence that CD4+CD25+ Tregs play a role in autoimmune disease

Animal models

Studies into the cause of autoimmune disease have demonstrated that it can be induced in normal animals by the elimination of particular subpopulations of $CD4^+$ T cells and that the reconstitution of the eliminated population results in the prevention of that autoimmune disease. In an early study it was demonstrated that spleen cells, from which certain T cell subsets had been removed, could cause the development of organ-specific autoimmune diseases, such as oophoritis, gastritis, thyroiditis, and orchitis, when transferred to nude mice.[54] A T cell subpopulation, $Thy\text{-}1^+Lyt\text{-}1, 2, 3^-$ cells, were shown to be responsible for the autoimmune disease induction. A different T cell subpopulation, $Lyt\text{-}1^+, 2, 3^-$ cells, were shown to have suppressive activity and were able to inhibit disease induction when co-transferred with the Lyt-1, 2, 3^- cells. Another early study using an autoimmune thyroiditis murine model reported that $Lyt\text{-}1^{dull}$ $L3T4^+$ T cells were responsible for the induction of thyroiditis and $Lyt\text{-}1^{bright}$ regulatory T cells were able to inhibit this induction.[55] In another murine study it was reported that $CD4^+$ neonatal splenocytes and $CD4^+CD8^-$ adult thymocytes were required for induction of autoimmune oophoritis and gastritis. Adult spleen cells did not elicit disease, but they prevented disease when co-transferred with neonatal spleen cells.[56]

Rat models have also been used to investigate the causes of autoimmune disease. Induction of autoimmune diabetes, insulitis, and thyroiditis in athymic rats by major histocompatibility complex compatible spleen cells was facilitated by prior *in vivo* depletion of $RT6.1^+$ regulatory T cells.[57] In another rat model it was reported that $CD45RB^{high}CD4^+$ T cells were responsible for the development of a severe wasting disease with inflammatory infiltrates in liver, lung, stomach, thyroid, and pancreas in athymic rats.[58] In contrast, $CD45RB^{low}CD4^+$ T cells did not induce disease. Animals given unfractionated $CD4^+$ T cells, containing approximately two-thirds $CD45RB^{high}$ and one-third $CD45RB^{low}$, were protected from the wasting disease, and the incidence of organ-specific inflammation was reduced, suggesting that the $CD45RB^{low}CD4^+$ T cells were able to inhibit the pathogenic $CD45RB^{high}$-$CD4^+$ T cells.

Sakaguchi *et al.* were one of the first groups to demonstrate that $CD4^+CD25^+$ Tregs have a clear role in regulating autoimmune disease.[4] They reported that the elimination of $CD4^+CD25^+$ T cells by use of an anti-CD25 monoclonal antibody resulted in a wide range of organ-specific and systemic autoimmune diseases, such as thyroiditis and gastritis, as well as graft-versus-host disease-like wasting disease in normal mice. Reconstitution of mice with $CD4^+CD25^+$ T cells prevented the development of these autoimmune diseases. In a different study it was demonstrated that transfer of $CD4^+CD25^+$ Tregs from normal mice, which had had their anergic state and suppressive function abrogated by addition of high levels of IL-2 or anti-CD28 antibody, produced a variety of autoimmune diseases in syngeneic athymic nude mice.[6] Similar murine studies support the observation that $CD4^+CD25^+$ T cells can prevent the development of multiple-organ autoimmune disease.[10,59,60]

In terms of specific autoimmune disease $CD4^+CD25^+$ Tregs have been reported to play an important role in the regulation of diabetes. Both B7.1 (CD80)/B7.2 (CD86)-deficient and CD28-deficient NOD mice, which have decreased numbers of $CD4^+CD25^+$ T cells, develop spontaneous type 1 diabetes.[61] Transfer of the $CD4^+CD25^+$ Treg subset from control NOD mice into CD28-deficient mice can delay and even prevent diabetes from developing. Similar finding were made in a rat model of autoimmune diabetes.[62]

As already mentioned *scurfy* mice develop fatal autoimmune lymphoproliferative disease.[63] *Scurfy* mice lack $CD4^+CD25^+$ Tregs due to mutations in the Foxp3 gene.[28] Fontenot *et al.* reported that the adoptive transfer of $CD4^+CD25^+$ Tregs into neonatal *scurfy* mice prevented the development of the lymphoproliferative disease.[31]

In all of these animal models the development of disease involves manipulation of T cell homeostasis and is inhibited by a subpopulation of normal $CD4^+$ T cells, mainly the $CD4^+CD25^+$ Treg population.

Human studies

Studies of $CD4^+CD25^+$ Tregs and their role in hu-

man autoimmune disease are less abundant than animal model studies. Human studies are limited to investigating frequencies and suppressive functions of $CD4^+CD25^+$ Tregs in patient samples. Unlike animal models, it is not currently possible to deplete or reconstitute Treg populations in human patients.

As already mentioned a disease, IPEX, similar to that found in *scurfy* mice, has been discovered in humans. IPEX is a rare, aggressive and fatal disease found in male children. It usually causes inflammatory bowel disease, neonatal diabetes, thyroiditis, and severe infection. A number of different mutations in the human Foxp3 gene are responsible for the development of this disease.[29,30] A recent study has shown that the number and phenotype of $CD4^+CD25^+$ T cells from IPEX patients are comparable to those of normal donors.[64] However, functional analysis revealed that $CD4^+CD25^+$ Tregs may either be normally suppressive or impaired to different degrees depending on: (a) the genotype of the target cells; (b) the type of *Foxp3* mutation; and (c) the strength of TCR activation. The authors concluded that *Foxp3* mutations in IPEX patients result in a range of biological abnormalities, leading to defective $CD4^+CD25^+$ Tregs and effector T cells, but not necessarily to a lack of differentiation of $CD4^+CD25^+$ Tregs.

Multiple sclerosis (MS) is an autoimmune disease thought to be mediated by T cells recognizing myelin protein peptides. A study by Viglietta *et al.* has shown that, although there is no difference in the frequency of $CD4^+CD25^+$ T cells in MS patients compared to healthy controls, there is a significant decrease in the suppressive activity of $CD4^+CD25^+$ Tregs from MS patients.[65] They demonstrated, through co-mixing experiments, that the decrease in regulatory activity was due to a defect in the $CD4^+CD25^+$ Treg population rather than the responder $CD4^+CD25^-$ T cells being refractory to suppression.

Autoimmune polyglandular syndromes (APS) are a group of disorders in which multiple endocrine glands are damaged by an autoimmune mechanism. There are two types of APS: APS type I is caused by loss of central tolerance; whereas little was know of the etiology of APS type II (APS-II). It had previously been reported that in several murine models, depletion of $CD4^+CD25^+$ Tregs caused a syndrome resembling human APS-II with multiple endocrinopathies. Kriegel *et al.* therefore hypothesized that loss of active suppression in the periphery could be a major cause of APS-II.[66] They demonstrated that there was no difference in the frequency or surface phenotype or apoptosis rates of $CD4^+CD25^+$ Tregs in APS-II compared to controls. Like MS, it was shown that $CD4^+CD25^+$ Tregs from APS-II patients were defective in their suppressive activity and that this defect was persistent and not due to responder cell resistance.

Myasthenia gravis (MG) is an autoimmune disease which is characterised by fluctuating, sometimes fatal, muscle weakness. The thymus is believed to be the initiation site of pathogenesis. It has been reported that MG patients have normal numbers of $CD4^+CD25^+$ thymocytes but these Tregs have a severe functional defect in their regulatory activity together with a decreased expression of Foxp3.[67]

Type 1 diabetes is a T cell–mediated autoimmune disease. It has been reported that although levels of $CD4^+CD25^+$ T cells are normal in patients with recent-onset adult type 1 diabetes, the suppressive activity of the Tregs in this population is reduced compared with control subjects.[68] Interestingly, a higher proportion of the $CD4^+CD25^+$ T cells co-express the early activation marker CD69 and intracellular CTLA-4.

Together, the above studies have reported that in MS, APS-II, MG and type 1 diabetes there is no difference in the frequency of $CD4^+CD25^+$ Tregs in patient groups as compared to normal controls. The differences arise in the suppressive activity of $CD4^+CD25^+$ Tregs: the $CD4^+CD25^+$ Tregs from each patient group had reduced suppressive activity when compared to the normal control groups. Other studies have reported a decrease in $CD4^+CD25^+$ Treg frequency in human autoimmune disease as compared to normal controls: autoimmune lymphoproliferative syndrome[69]; SLE[70,71]; DiGeorge syndrome[72]; and Kawasaki disease.[73] The Kawasaki disease patients also had reduced mRNA expression levels of Foxp3, GITR and CTLA-4.[73] It has recently been reported that $CD4^+CD25^+$ Tregs from active SLE patients have decreased suppressive activity and reduced Foxp3 mRNA and protein levels as compared with normal controls and patients with inactive SLE.[74] There are also conflicting reports as to the frequencies of $CD4^+CD25^+$ Tregs in autoimmune disease. The above mentioned study on type 1 diabetes reported normal levels of $CD4^+CD25^+$ Tregs, whereas a different study reported that $CD4^+CD25^+$ Treg frequencies were lower in both newly diagnosed and

long-term type 1 diabetes patients as compared to normal controls.[75]

CD4+CD25+ Tregs and rheumatoid arthritis

RA is a chronic inflammatory autoimmune disease affecting about 0.8% of the UK adult population. Progression can be very rapid, and is characterised by swelling of the synovium and damage of the cartilage and bone around the joints, ultimately leading to joint destruction. Any joint may be affected but RA more commonly starts in the hands, feet and wrists. The exact etiology and development of RA is not fully elucidated, but the presence of inflammatory cytokines is thought to play a key role in the induction and maintenance of this disease. A study investigating the natural immune response against a candidate autoantigen in RA, human cartilage glycoprotein-39 (HC gp-39), has found that in normal controls the response is biased towards a regulatory (IL-10) phenotype, whereas in RA patients it is biased towards a pro-inflammatory Th1 (IFN-γ) phenotype.[76] This suggests that in a normal state the presence of HC gp-39-specific T cells may have an inhibitory effect on inflammatory responses in areas where HC gp-39 is present. In RA there appears to be a breakdown of tolerance, which causes the response to shift from a regulatory one to a pro-inflammatory one.

Animal models

There are two frequently studied murine models of RA: collagen-induced arthritis (CIA)[77,78] and antigen-induced arthritis (AIA)[79]. CIA is induced through immunisation of mice with bovine type II collagen (CII) emulsified in Freund's complete adjuvant. This leads to production of CII-specific antibodies, which are necessary for disease induction. Therefore, CIA is an antibody-mediated, B cell-dependent autoimmune disease. CIA is not identical to RA, but they do share many key features, such as synovitis, erosions of both bone and cartilage, and class II major histocompatibility complex–linked susceptibility. CIA is mediated by both cellular and humoral immune responses, whereas AIA is dependent on cell-mediated immunity, with a minor contribution of humoral immunity. AIA is induced by pre-immunisation of mice with methylated bovine serum albumin (mBSA) in complete Freund's adjuvant. The knee joint is then intra-articularly injected after 21 days with mBSA in saline. This results in a T cell-dependent disease in which an initial acute inflammatory reaction is followed by chronic disease, characterised by synovial hyperplasia, infiltration of mononuclear cells, and cartilage and bone destruction. The histopathological changes are similar to those that occur in RA. The AIA model results in 100% incidence of arthritis and has a major advantage over CIA in that the time point of induction of arthritis is known.

Morgan *et al.* used the CIA murine model to demonstrate that depletion of $CD4^+CD25^+$ Tregs prior to CII immunisation greatly increased both the severity and incidence of the disease and was associated with an increase in CII-specific antibodies.[80] Adoptively transferring $CD4^+CD25^+$ Tregs into $CD25^+$-depleted mice reversed severity of the disease. In a later paper, the same group reported that $CD4^+CD25^+$ Tregs can be used therapeutically in CIA.[81] They adoptively transferred $CD4^+CD25^+$ Tregs into mice during the early stage of CIA and showed that disease progression was markedly slowed despite a lack of reduction in systemic CII-specific T and B cell responses. $CD4^+CD25^+$ Tregs were traced to the synovial tissue in affected joints, indicating that these cells may modulate inflammation locally in the joint. $CD4^+CD25^+$ Tregs and their role in AIA have also been investigated.[82] Depletion of $CD25^+$ cells in immunised animals before arthritis induction led to an exacerbation of disease. Transfer of $CD4^+CD25^+$ Tregs into immunised mice at the time of induction of AIA decreased the severity of disease but was not able to cure established arthritis. Again, like the previous study, $CD4^+CD25^+$ Tregs were found to accumulate in the inflamed joint. It would appear that in murine models of RA $CD4^+CD25^+$ Tregs can migrate to sites of inflammation and play an important role in preventing the induction of disease, but are unable to cure established disease.

Human studies

Frequencies of $CD4^+CD25^+$ Tregs in the periphery and synovial fluid

A number of studies have examined $CD4^+CD25^+$ Tregs in human patients with RA and other types of inflammatory arthritis. As the two previously mentioned murine studies have shown that $CD4^+CD25^+$ Tregs can migrate to sites of inflammation it is important to investigate $CD4^+CD25^+$ Tregs from inflamed joints, as well as the periphery. Several studies have reported an enrichment of $CD4^+CD25^+$ Tregs in the synovial fluid (SF) of patients with RA and other types of inflammatory

arthritis.[83-89] This enrichment was demonstrated to be irrespective of disease duration, severity or drug treatment.[84,85] The SF $CD4^+CD25^+$ Tregs did display suppressive activity in both terms of proliferation and cytokine production.[83-89]

There is controversy surrounding the frequency of $CD4^+CD25^+$ Tregs in the peripheral circulation of inflammatory arthritis patients compared to normal controls: some studies report normal numbers;[83,86,87,90] some report an increase[88]; and others report a decrease.[84,89,91] Some of this variability may be explained by differences in disease stage and therapy. For example it has been shown that early active RA patients who had received no disease--modifying therapy had a smaller proportion of $CD4^+CD25^+$ Tregs in the peripheral blood than controls, whereas stable, well-controlled RA patients who were receiving therapy had similar numbers of $CD4^+CD25^+$ Tregs to controls.[89] There are also differences in the phenotypic definition of naturally-occurring Tregs in the above mentioned studies. Some studies used $CD4^+CD25^+$ Tregs, which included total $CD25^+$ T cells, whereas some used only the $CD4^+CD25^{bright}$ Tregs.

$CD4^+CD25^+$ Treg frequencies have also been found to be altered in JIA.[92] JIA and RA, although not the same disease, do have similar mechanisms of disease pathogenesis and clinical presentation. Persistent oligoarticular JIA (pers-OA JIA) is a subtype of JIA with a relatively benign, self-remitting course, while extended oligoarticular JIA (ext-OA JIA) is a subtype with a much less favorable prognosis. It has been shown that patients with pers-OA JIA had a higher frequency of $CD4^+CD25^{bright}$ Tregs in the peripheral blood than ext-OA JIA patients. The numbers of SF $CD4^+CD25^{bright}$ Tregs were equal in both groups and these cells displayed suppressive activity. Interestingly, the pers-OA JIA patients displayed a higher frequency of SF $CD4^+CD25^{int}$ T cells, which were found to express Foxp3 mRNA and also displayed suppressive activity. It would therefore appear that there are more $CD4^+CD25^+$ $Foxp3^+$ Tregs in the SF of pers-OA JIA patients. Therefore, $CD4^+CD25^+$ Tregs may play a role in determining disease outcome by directing the disease course to either a favorable or unfavorable one. The less favorable ext-OA JIA may be due to $CD4^+CD25^+$ Tregs being unable to migrate to or expand at sites of inflammation.

Characterisation of synovial fluid $CD4^+CD25^+$ Tregs

In RA and other inflammatory arthritis there appears to be two compartments of $CD4^+CD25^+$ Tregs: those in the peripheral blood and those at the sites of inflammation, usually in the SF. One important question is whether these two populations of $CD4^+CD25^+$ Tregs are phenotypically and functionally similar. It has been reported that the majority of $CD4^+CD25^+$ Tregs from inflamed joints are activated $CD45RO^+$ memory cells expressing a variety of activation markers, such as HLA-DR and CD71[83] and are phenotypically dissimilar to peripheral blood $CD4^+CD25^+$ Tregs: SF $CD4^+CD25^+$ Tregs display a higher expression of CTLA-4 [83,87,88,92], GITR, CD69[87;88;92], OX40[87,88], HLA-DR[88,92], $CD25$[87], Foxp3 (mRNA)[92], and CD71 with a lower expression of CD62L.[83] These studies suggest that the $CD4^+CD25^+$ Tregs may undergo maturation in the joint. In correlation with this activated, mature phenotype, the SF $CD4^+CD25^+$ Tregs show increased regulatory activity compared with peripheral blood $CD4^+CD25^+$ Tregs.[43,88,92]

Are CD4+CD25+ Tregs defective in RA?

Another very important question is why, when $CD4^+CD25^+$ Tregs accumulate in joint and show enhanced suppressive activity, is RA able to progress? One study has reported that the $CD4^+CD25^-$ responder T cells in the SF are activated and as such are less susceptible to suppression by $CD4^+CD25^+$ Tregs than their resting counterparts in the peripheral blood.[88] Importantly, this decreased susceptibility to suppression was observed when $CD4^+CD25^+$ Tregs from either peripheral blood or SF were used. Therefore, although $CD4^+CD25^+$ Tregs can migrate to inflamed areas and do possess suppressive activity, their ability to suppress arthritis may be limited by local responder T cells having a reduced susceptibility to regulation. It should be noted that a different study has also investigated the ability of $CD4^+CD25^+$ Tregs to suppress both peripheral blood and SF responder cells and has shown the degree of suppression was similar in one patient but actually lower for the peripheral blood responders in another patient.[83] These differences could be explained by differences in separation of the Treg populations: van Amelsfort *et al.* used MACS separation to obtain $CD4^+CD25^+$ Tregs, which included total $CD25^+$ T cells, whereas Cao *et al.* used a FACS-sort to obtain only the $CD4^+CD25^{bright}$ Tregs. The van Amelsfort study is in keeping with a previous study which reported that strongly activated $CD4^+$ responder T cells are resistant to regulation by $CD4^+CD25^+$ Tregs, while weak-

ly stimulated $CD4^+$ cells are sensitive to suppression.[93] Therefore, resistance to suppression is dependent upon the strength and duration of the stimulus: the stronger the TCR signal, the quicker and more fully the responder cells become refractory to suppression.

Ehrenstein *et al.* have reported that $CD4^+CD25^+$ Tregs derived from peripheral blood of patients with active RA are defective in their ability to suppress pro-inflammatory cytokine production, but not proliferation.[90] As the presence of pro-inflammatory cytokines is very important in the development of RA this observation is of great significance. However, it should be noted that this finding differs from other studies using cells from SF.[84-86,88]

Another explanation as to why RA is able to progress is the cytokine profile in the joint and the effect this has on $CD4^+CD25^+$ Tregs. The cytokine milieu of the RA joint includes many pro-inflammatory cytokines, such as TNF-α, IL-1, IL-6, GM-CSF, anti-inflammatory cytokines, such as IL-10 and TGF-β, and cytokine inhibitors such as IL-1RA and soluble TNF-R (reviewed in McInnes and Schett, 2007).[94] It would appear that there is imbalance between pro-inflammatory and anti-inflammatory cytokines in the joint. Therefore, although IL-10, for example, is expressed in the joint it is not present in a high enough concentration to mediate counter-regulatory activity against the dominant pro-inflammatory cytokine milieu.

It has been reported that the SF cytokine profile in early RA is transient and distinctly different from established RA.[95] The levels of a range of T cell, macrophage and stromal cell-related cytokines, including IL-2, IL-4, IL-13, IL-17, IL-15, bFGF and EGF, are significantly increased in early RA patients. Therefore, the disease stage may have an influence on the pro-inflammatory/anti-inflammatory cytokine balance and play an important role in disease development to persistent RA. For example, as has already been mentioned, both $CD4^+CD25^+$ Treg anergy and suppressive function can be overcome by the addition of high levels of IL-2.[5-7] This may play a significant role in the inhibition of suppression by $CD4^+CD25^+$ Tregs in early stages of the disease, which would allow responder cell activation and creation of a pro-inflammatory environment resulting in progression to established RA and joint destruction.

A recent study by van Amelsfort *et al.* has demonstrated that activated monocytes from the SF of RA patients produce both IL-7 and TNF-α and these two cytokines abrogate the suppressive activity of $CD4^+CD25^+$ Tregs.[96] It was suggested that the effect of IL-7 may be to render the responder T cells resistant to suppression, since the $CD4^+CD25^+$ Tregs have very low expression of the IL-7 receptor (CD127). IL-6 is also found in high levels in SF from the joints of patients with active RA.[97] Pasare and Medzhitov have demonstrated that IL-6, secreted by DCs upon TLR stimulation, has an effect on responder T cells and renders them resistant to $CD4^+CD25^+$ Treg suppression.[98] In the above mentioned study by van Amelsfort *et al.* IL-6 was reported to have no effect on the suppressive activity of $CD4^+CD25^+$ Tregs.[96] This may be explained by the fact that Pasare and Medzhitov have demonstrated that IL-6 alone is not sufficient for the inhibition of suppression and another unknown TLR-induced factor is also needed.[98] This unknown, TLR-induced factor may be missing from the SF in RA joints. Therefore, the interaction between $CD4^+CD25^+$ Tregs and activated monocytes in the joint might lead to diminished suppressive activity of $CD4^+CD25^+$ Treg cells, which contributes to the chronic inflammatory state that is RA.

The balance of evidence suggests that $CD4^+CD25^+$ Tregs *per se* may not have defective suppressive activity in RA. The defect in peripheral tolerance may reflect the cytokine environment in the joint, which may render responder T cells resistant to suppression and/or have a direct effect on $CD4^+CD25^+$ Tregs by inhibiting their suppressive activity.

Therapeutic potential of CD4+CD25+ Tregs in RA

Anti-TNF-α treatment and CD4+CD25+ Tregs

The animal models of autoimmune disease discussed above have demonstrated that adoptive transfer of $CD4^+CD25^+$ Tregs can prevent or even cure certain autoimmune diseases. One very important question is: can $CD4^+CD25^+$ Tregs be used or manipulated as a therapy for use in RA? Present RA treatments and their effect on $CD4^+CD25^+$ Tregs have been studied. In the previously mentioned study by Ehrenstein *et al.* in which it was reported that $CD4^+CD25^+$ Tregs from RA patients cannot suppress pro-inflammatory cytokine production, it was demonstrated that treatment with anti-TNF-α (infliximab) restored the capacity of $CD4^+CD25^+$ Tregs to inhibit cytokine produc-

tion.[90] The clinical response to infliximab, but not conventional methotrexate treatment, correlated with an increased frequency of peripheral blood $CD4^+CD25^+$ Tregs, which in turn correlated with a reduction in C-reactive protein (a measure of disease activity). It was reported that TNF-α does not have a direct effect on $CD4^+CD25^+$ Tregs as exposure to a gradient of TNF-α concentration did not affect viability or function of these cells. However, in opposition to this finding it has been reported that TNF inhibits the suppressive activity and downregulates Foxp3 expression in both naturally--occurring $CD4^+CD25^+$ Tregs and TGF-β 1–induced $CD4^+CD25^+$ Tregs.[99] Treatment with infliximab was shown to increase Foxp3 expression by $CD4^+CD25^+$ Tregs and restore their suppressive activity. It has also been reported that $CD4^+CD25^+$ Tregs are apoptosis-prone[3,100] and that spontaneous apoptosis of $CD4^+CD25^+$ Tregs from active RA patients was increased, whereas the absolute number of peripheral blood $CD4^+CD25^+$ Tregs was reduced when compared to controls.[91] After infliximab treatment spontaneous apoptosis was reduced in the RA patients and $CD4^+CD25^+$ Treg numbers were equivalent to those of normal controls. The alteration and reversal in both spontaneous apoptosis and numbers of $CD4^+CD25^+$ Tregs was found to correlate with RA disease activity, as measured by C-reactive protein. This would seem to suggest that TNF--α can affect $CD4^+CD25^+$ Tregs and infliximab treatment reverses this. The Ehrenstein group has since gone on to demonstrate that infliximab therapy gives rise to a $CD4^+CD25^{hi}FoxP3^+$ Treg population, which mediates suppression via TGF-β and IL--10.[101] *In vitro* studies have shown that this Treg population was induced from $CD4^+CD25^-$ T cells and the process was dependent on TGF-β, suggesting that these Tregs may resemble Th3 cells. These induced Tregs can be distinguished from naturally-occurring $CD4^+CD25^+$ Tregs by their lack of CD62L expression. It was reported that the induced $CD62L^-$ Tregs have very potent suppressor activity, but the naturally-occurring $CD62L^+$ T regs remain defective in infliximab-treated patients. Therefore, the induction of $CD62L^-$ Tregs could be used as a viable therapeutic tool for the restoration of tolerance in RA patients.

Adalimumab is another anti-TNF-α therapy used in the treatment of RA. Infliximab is a chimeric anti-TNF-α monoclonal antibody, whereas adalimumab is a fully human anti-TNF-α antibody. They are both able to block the interaction of TNF--α with its receptors; however their efficacy and side effects are not the same. Adalimumab has been found to increase numbers and improve the function of $CD4^+CD25^+$ Tregs in the peripheral blood of RA patients at day 15 of treatment.[102] However, after 6 months of treatment $CD4^+CD25^+$ Treg numbers had fallen but remained higher than pretreatment levels. In opposition to this another report studying the effect of adalimumab on $CD4^+CD25^+$ Tregs from RA patients demonstrated that the treatment had no effect on these cells.[103] Collectively, these studies suggest that TNF-α blockade, in addition to its anti-inflammatory effects, may play an important regulatory role in the amelioration of RA. This regulation may be through the induction of a $CD62L^-$ Treg population. It is of note that withdrawal of anti-TNF-α therapy from RA patients generally causes them to relapse[104], although a small study performed in patients with early RA suggested regulatory effects that lasted beyond the duration of treatment.[105] Therefore, patients who respond to therapy may require on-going treatment to maintain their response in the long term. It would appear that, at least in established disease, anti-TNF-α therapy provides immunosuppression rather than immunomodulation and, while this treatment helps control RA progression, it does not provide a cure for RA. Further investigation is required of its potential effects in patients with RA of recent onset.

Autologous stem cell transplantation and CD4+CD25+ Tregs

Autologous stem cell transplantation (ASCT) has been used in children to treat JIA with some promising results: 53% of the children achieved complete drug-free remission, some of whom had previously failed treatment with all licensed therapies, including anti-TNF-α; 18% showed a partial response; and 21% were resistant to the therapy.[106,107] In a recent study it was shown that the good outcome from ASCT in JIA may be linked to the restoration of immunologic self-tolerance.[108] There were reduced numbers of $CD4^+CD25^+$ Tregs before ASCT as compared to normal controls. After ASCT there was an increased frequency of Foxp3 expressing $CD4^+CD25^+$ Tregs with numbers returning to normal levels. It was shown that this recovery was due to a preferential homeostatic expansion of $CD4^+CD25^+$ Tregs during the lymphopenic phase of immune-reconstitution. It was also shown that ASCT induces reprogramming of autoimmune T

cells from a pro-inflammatory phenotype (IFN-γ and T-bet) to a tolerant phenotype (IL-10 and GATA-3 high). These data suggest that the restoration of the $CD4^+CD25^+$ immune regulatory network and reprogramming of autoreactive T cells is possible in some human autoimmune diseases.

Ex vivo generation of CD4+CD25+ Tregs

If $CD4^+CD25^+$ Tregs are to be used as a therapeutic tool it is important that these cells can be generated *ex vivo*. Several studies have demonstrated that human $CD4^+CD25^+$ Tregs can be expanded to large numbers when stimulated via CD3 and CD28 in the presence of high doses of IL-2.[109,110] The expanded $CD4^+CD25^+$ Tregs remained anergic and retained their suppressive activity. It has been reported that in the NOD murine model of diabetes the ability of expanded $CD4^+CD25^+$ Tregs to suppress diabetes in pre-diabetic and diabetic mice *in vivo* was greatly enhanced by using an autoantigen-specific stimulus.[111] Therefore, in contrast to the above mentioned study by Frey *et al.*, in which it was demonstrated that polyclonal $CD4^+CD25^+$ Tregs alone appear unable to counteract established and ongoing acute or chronic inflammation[82], expanded antigen-specific $CD4^+CD25^+$ Tregs cannot only suppress the development of disease but can also reverse it after disease onset. This provides hope that antigen-specific $CD4^+CD25^+$ Tregs can be used as a cellular therapy for autoimmune disease. These Tregs would only be activated in the target organ, thus focusing their effects. The auto-antigens involved in RA, once they are identified, could be used to increase the potency of $CD4^+CD25^+$ expanded Tregs. However, administration of large numbers of $CD4^+CD25^+$ Tregs should be approached with caution. The presence of such high numbers of regulatory cells may create an environment that favours development of more regulatory cells via infectious tolerance. High numbers of $CD4^+CD25^+$ Tregs could then cause non-antigen-specific bystander suppression, leaving the recipient vulnerable to infections or tumours.

In vivo expansion of CD4+CD25+ Tregs

There are practical and technical difficulties associated with the *ex-vivo* expansion of $CD4^+CD25^+$ Tregs. Any therapy destined for human use must be developed to a clinical-grade, compatible with good manufacturing practice requirements. Therapies which allow the *in vivo* expansion of $CD4^+CD25^+$ Tregs may be a better option. In two separate studies, anti-CD3 monoclonal antibodies were used to treat patients with recent-onset type 1 diabetes mellitus.[112,113] Treatment in both studies maintained or improved insulin production, which reduced the requirement for insulin doses for at least 12 months. No severe side effects occurred. In one of the studies clinical responses were associated with a change in the ratio of $CD4^+$ T cells to $CD8^+$ T cells.[112] This group went on to demonstrate that the increase in $CD8^+$ T cells was due to the induction of a population of $CD8^+CD25^+Foxp3^+$ Tregs, which were able to regulate autologous, antigen-specific $CD4^+$ T cells in a cell contact–dependent manner.[114] They had previously shown that treatment with anti-CD3 monoclonal antibody also induces $CD4^+IL\text{-}10^+$ T cells, which may also play a role in tolerance.[115] A murine study has demonstrated that a combination of anti-CD3 monoclonal antibody with intranasal proinsulin can reverse recent-onset diabetes with higher efficacy than anti-CD3 monoclonal antibody alone and lead to the expansion of insulin-specific $CD4^+Foxp3^+$ Tregs producing IL-10, TGF-β, and IL-4.[116] These cells could transfer tolerance to immunocompetent recent-onset diabetic mice and could also suppress autoaggressive CD8 responses. Therefore, anti-CD3 monoclonal antibody therapy could be a promising therapy for RA, especially if used in combination with an immunisation protocol, e.g. oral or intranasal immunisation with autoantigens.

CD28 superagonists are also capable of activating and preferentially expanding Tregs in various experimental animals and have been shown to have therapeutic effects in various models of autoimmunity (reviewed in Beyersdorf et al., 2005).[117] It should be noted, however, that in a recent phase I clinical trial the use of a CD28 superagonist caused a cytokine storm, characterised by a rapid induction of pro-inflammatory cytokines resulting in a systemic inflammatory response.[118] Therefore, the use of CD28 superagonists, while showing promising results in animal models, should be approached with great caution when considered for the treatment of human autoimmune diseases.

New therapies and CD4+CD25+ Tregs

There are several promising new approaches for tolerance induction, which have been studied in humans. The first used a mucosal peptide-specific immunotherapy to induce immunomodulation.[119] It was demonstrated that dnaJP1, a heat

shock protein-derived peptide, induces *in vitro* proliferative and pro-inflammatory T cell responses in a proportion of RA patients. Oral administration of this peptide over a six month treatment period, however, induced immune deviation from a pro-inflammatory (IFN-γ and TNF-α) to a regulatory (IL-4 and IL-10) response. As the peptide was orally administered there was the possibility that T cells may have been clonally deleted, however, this was ruled out. It was found that Foxp3 expression by $CD4^+CD25^+$ Tregs was increased suggesting that the treatment caused a shift from a pathogenic T cell phenotype to regulatory one although no clinical correlates were reported in this small study. In a different study, T cell vaccination was used to induce regulatory immune responses in RA patients.[120] Autologous pathological synovial T cells were rendered inactive by irradiation, and used for vaccination. This led to induction of IL-10-producing $CD4^+Foxp3^+$ Tregs and $CD8^+$ cytotoxic T cells specific for the T cell vaccine. The observed regulatory immune responses collectively correlated with clinical improvement in treated patients.

Other potential therapies for the treatment of RA have been investigated in murine models. These include the use of immunomodulatory neuropeptides, vasoactive intestinal peptide (VIP)[121], andrenomedullin[122] and urocortin[123], to induce IL-10/ /TGF-β-producing $CD4^+CD25^+Foxp3^+$ Tregs, which are able to suppress and ameliorate the progression of CIA; the use of the candidate autoantigen, BiP, to induce IL-4-producing Tregs, which are able to suppress established CIA[124]; and the use of immunoregulatory $CD8^+$ T cells to suppress rheumatoid synovitis.[125]

Tolerogenic DCs may be another effective method of inducing Tregs, although not necessarily naturally-occurring Tregs. For example, it has been shown that VIP induces the generation of IL-10--producing tolerogenic DCs with the capacity to generate $CD4^+$ and $CD8^+$ Tregs.[126] The $CD4^+$ Tregs resembled Tr1 cells, i.e. they were anergic and produced IL-10 and TGF-β, whereas the majority of CD8 Tregs were IL-10-producing $CD8^+CD28^-$ T cells. Both $CD4^+$ and $CD8^+$ Tregs suppressed antigen-specific Th1-mediated responses. This therapy may be more successful in treating RA if the tolerogenic DCs are loaded with a target autoantigen, which when presented to naïve T cells could induce the development of antigen-specific Tregs.

Genetic manipulation of T cells to generate Tregs is also a promising therapeutic tool. It was reported to be successful in a murine model of type 1 diabetes.[127] Ectopic expression of Foxp3 by retroviral transduction was shown to confer a suppressor phenotype to naive $CD4^+$ T cells. Only antigen-specific Foxp3-transduced T cells and not polyclonal Foxp3-transduced T cells were effective in stabilising and reversing established disease.

In summary, there are many avenues for novel therapeutic treatments for RA and other autoimmune diseases involving $CD4^+CD25^+$ Tregs. However, more work is required to establish whether $CD4^+CD25^+$ Tregs can be used as a therapy for RA to not only decrease the severity and progression of disease but also to cure established disease. As it would appear that the cytokine environment in the joint plays a very important role in the inhibition of peripheral tolerance, any potential therapy for RA may need to take this into account. An appropriate regime may need not only to induce functioning Tregs but also to address the cytokine milieu of the joint.

Overall conclusions

$CD4^+CD25^+$ Tregs play a very important role in various autoimmune diseases. A number of animal studies have shown that elimination of $CD4^+CD25^+$ Tregs induces autoimmunity and that reconstitution of these cells inhibits it. Therefore, there is great potential to use these cells in a therapeutic regime for the treatment, and possible cure, of autoimmune disease, including RA. However, a number of issues must be addressed before a $CD4^+CD25^+$ Treg therapy can be considered for use in humans: 1) a $CD4^+CD25^+$ Treg-specific marker, or a combination of markers, such as Foxp3 and CD127, must be agreed upon and used to ensure the purity and correct identification of $CD4^+CD25^+$ Tregs; 2) it must be established that administration or manipulation of $CD4^+CD25^+$ Tregs will only regulate specific, localised targets and not cause systemic immunosuppression. This may require the derivation of antigen-specific T-regs, only possible when an autoantigen is clearly defined; 3) when designing a therapeutic regime it may be prudent to use $CD4^+CD25^+$ Tregs in combination with another treatment as administration or manipulation of $CD4^+CD25^+$ Tregs alone may not be enough to cure established disease.

The *in vivo* induction of Tregs using, for example, anti-CD3 antibodies, or autoantigenic pepti-

des, provides an equally appealing approach which currently appears closer to clinical application.

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DENDRITIC CELL SUBSETS: THEIR ROLES IN RHEUMATOID ARTHRITIS

Maria C. Lebre, Paul P. Tak

Resumo

As células dendríticas são capazes de influenciar várias classes de linfócitos (linfócitos B, células NK) e vários tipos de respostas T (Th1/Th2/Th17, células T reguladoras, deleção periférica de célúlas T). As células dendríticas não só foram identificadas na artrite reumatóide (AR) como também têm sido implicadas na etiopatogenia desta doença. Esta revisão sumariza os resultados obtidos até ao momento sobre a caracterização e função de várias populações de células dendríticas na AR. O efeito da terapêutica anti-TNF-α na função e fenótipo das células dendríticas é também discutido. Uma vez que a maioria dos trabalhos realizados em modelos animais de artrite utilizaram células dendríticas imuno-modificadas como veículo terapêutico da artrite, apresentamos vários exemplos de como estas células são capazes de induzir tolerância *in vivo*. Apesar do muito trabalho que tem sido sido efectuado até ao momento, fica ainda por estabelecer quais os papéis especificos de cada sub-população de células dendríticas na AR e em modelos animais de artrite. O conhecimento detalhado das funções especificas das células dendríticas na AR poderá, no futuro, perspectivar novas abordagens terapêuticas, usando imunoterapia com células dendríticas, de modo a diminuir a resposta autoimune .

Palavras-chave: Células Dendríticas; Artrite Reumatóide; Sangue; Líquido Sinovial; Membrana Sinovial.

Abstract

Dendritic cells (DC) are now known to influence many different classes of lymphocytes (T, B, NK cells) and many types of T cell responses (Th1/Th2/ /Th17, regulatory T cells, peripheral T cell deletion). In rheumatoid arthritis (RA) DC have been described and their roles in RA pathogenesis have been implicated. This review summarizes the data obtained so far concerning the functional characterization of several DC subsets in human RA. Moreover, the effect of TNF-α blockade on DC phenotype and function is also discussed. As most of the studies on DC in experimental arthritis have been conducted using (immunomodulated/tolerogenic) DC as tools to ameliorate experimental arthritis, we give some examples of how these cells may induce tolerance *in vivo*. Although a lot of work has been performed so far, the specific and functional roles of DC subsets in human RA and in CIA remain to be established. Achieving a detailed understanding of specific DC functions in RA holds potential for modulating DC for immunotherapy by down-regulating the autoimmune response.

Keywords: Dendritic Cells; Rheumatoid Arthritis; Blood; Synovial Fluid; Synovial Tissue.

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Introduction

Dendritic cells (DC) play a pivotal role in orchestrating of T cell immunity and tolerance due to their ability to stimulate naive T cells and direct effector cell function.[1] Human DC have been divided into two different subsets: myeloid (m) or conventional (c) DC and plasmacytoid (p) DC. These subsets constitute a heterogeneous population that consists of differences in tissue distribution, phenotype and function.[2]

Rheumatoid arthritis (RA) is a chronic inflammatory disease associated with the destruction of affected joints and represents one of the most common autoimmune-related diseases, affecting as much as 1% of Western populations. Clinically, RA manifests as a symmetric polyarthritis associated with swelling and pain in multiple joints, often ini-

tially occurring in the joints of the hands, wrists, and feet and is characterized by synovial hyperplasia and progressive joint destruction.[3] In this respect, there is a massive influx of T cells, B cells, fibroblast-like synoviocytes, macrophages and DC into the synovial tissue (ST).[4]

We here review the current advances on the potential roles of DC subsets in RA and review the recent studies concerning the functional and phenotypical characterization of DC subsets in human peripheral blood (PB), ST and synovial fluid SF. We will review the effect of current RA therapies (e.g. TNF-α blockade) on DC subset phenotype and function. Finally, we will summarize the role of DC in experimental arthritis and will give some examples about the use of these cells as therapeutic targets by either manipulating DC function *in vivo* or by transferring tolerogenic DC.

DC subsets

As mentioned above, DC constitute a heterogeneous population of antigen-presenting cells (APC) characterized by differences in tissue distribution, phenotype and function.[2] It has recently become possible to identify the different subsets within a DC population through their differential expression surface markers. Human PB DC have been divided into 4 distinct lineage negative HLA-DR$^+$ subsets: three of myeloid origin (all CD11c$^+$), CD1b/c$^+$, CD16$^+$ and CD141/BDCA3$^+$, and one of plasmacytoid origin CD123$^+$ pDC.[5] Myeloid DC (mDC) are also called conventional (c) DC, although this termed is more commonly used for murine DC. CD1c/BDCA1$^+$ mDC[6] are CD304/BDCA4$^-$, CD11c$^+$, CD45RO$^+$ and CD123^{low} and have the ability to produce IL-12 in response to bacterial compounds or CD40L, and require GM-CSF for survival.[7] From PB, CD1c$^+$ mDC are the most potent allogeneic T cell stimulators.[5] CD16$^+$ mDC are CD14$^-$, CD45RA$^+$, CD45RO$^+$, CD40$^+$[5] and selectively express CX3CR1 (fractalkine receptor) and Toll-like receptor (TLR).[8] Although this DC subset is capable of inducing allogeneic T cell proliferation, their stimulatory capacity is lower compared to monocyte-derived DC (mo-DC) and CD1c$^+$ mDC.[5] Recently, functional data on this subset became available. In comparison to CD1c$^+$ mDC, circulating CD16$^+$ mDC secrete 10- to 100-fold higher amounts of particularly TNF-α.[9] A minor population of CD141/BDCA3$^+$ mDC in peripheral blood has been described but not yet well functionally characterized.[6] However, it has been reported that BDCA3$^+$ mDC specifically express CCR9, TLR3 and high levels of CD40.[8] The increased CD40 expression by the CD16$^+$ and BDCA-3$^+$ mDC subsets possibly reflects a continuum of activation. CD123^{high} pDC are CD303/BDCA2$^+$, CD304/BDCA4$^+$, CD11c$^-$, CD45RA$^+$[6], and require the presence of IL-3 for survival.[10] When pDC are exposed to immune complexes consisting of anti-double stranded DNA or upon viral or bacterial infection, high amounts of type I IFN, IFN-α and IFN-β are produced.[11,12]

Another DC subset, that is absent in the peripheral blood, is the CD1a$^+$ mDC. This DC subset originates from blood-derived CD14$^+$ monocytes and is called mo-DC. The ability of monocytes to differentiate into DC was originally demonstrated by Sallusto and Lanzavecchia,[13] who reported the generation of DC from human peripheral monocytes after *in vitro* culture with GM-CSF + IL-4. Over the last 10 years, this method has prompted numerous studies on human DC that were previously hampered by the difficulties in working with *ex vivo*-isolated human DC, and it has proven to be an extremely powerful tool for the study of human DC differentiation and maturation processes.[14]

DC subsets in RA

Since the discovery of changes in the DC subsets in other autoimmune diseases (e.g. systemic lupus erythematosus, SLE),[15] various studies have been performed that addressed both local and systemic levels of DC subsets in RA.

DC have been identified in rheumatoid ST and SF by several groups over the last 15 years, and their origin, function, and potential role in the pathogenesis are not fully understood.[16] One of the first reports described an mDC population (CD33$^+$CD14$^-$) present in inflamed RA synovium, with a fully differentiated phenotype that expressed high levels of class I and II MHC and T cell costimulatory molecules.[17] In view of these observations the authors suggested that these mature mDC might help to perpetuate the inflammation by activation of autoreactive T cells present in the inflamed RA synovium. This report was just the beginning of a cascade of studies that investigated the role of DC in rheumatic diseases.

Myeloid DC in RA

CD1c+BDCA1+ mDC

As stated above, CD1c+ mDC are able to produce IL-12p70 in response to TLR ligands and CD40L.[7] Recently, CD1c+ mDC have been associated with induction of chemotaxis in response to TLR ligands, due to their capacity to produce elevated levels of various chemokines and in particular CXCL8/IL-8.[9]

In RA patients, CD1c+ mDC are present in different compartments: PB,[18] and SF[18] and ST[18](MC Lebre *et al.* Am J Pathol. *in press* and Figure 1, left panel). We have shown that circulating PB CD1c+ mDC were significantly reduced in RA compared to healthy controls with reduced CD62L expression. In RA SF, these cells were also present, but they displayed a more mature phenotype compared to PB. In addition, the number of PB circulating mDC in RA was significantly inversely correlated to C-reactive protein.[18] In RA ST these cells are in the vicinity of T cells and express the cytokines IL-12p70 and IL-23p19, important for the induction/expansion of the Th1 and Th17 T cell subsets, respectively (MC Lebre *et al.* Am J Pathol. *in press*). Importantly, most of the CD1c+ mDC present in RA ST are CD83- and DC-LAMP-. This DC subset may represent recently activated DC (because they express cytokines) that not yet up-regulated the maturation markers. Another explanation is that mature DC have migrated, upon activation, from the inflamed ST into the draining lymph node.

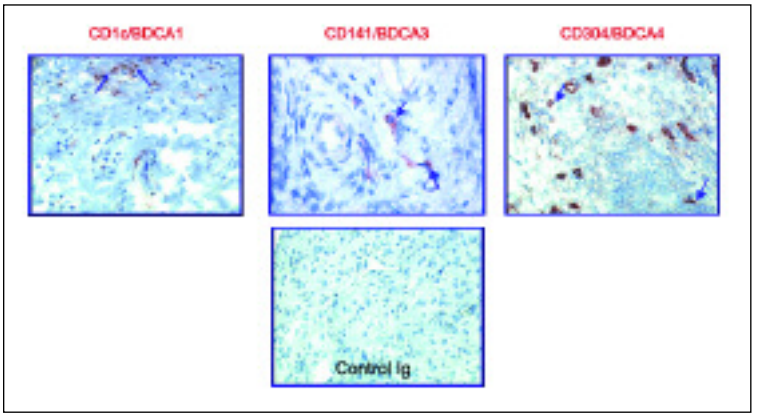

Figure 1. Expression of three DC subsets in RA synovial tissue. Immunohistochemistry staining of frozen sections from a representative RA ST using specific antibodies against CD1c/BDCA1 (mDC), CD141/BDCA3 (mDC) and CD304/BDCA4 (pDC). Arrows indicate red positive staining. Original magnification 250x.

CD1a+ mDC

PB CD14+ monocytes are able to differentiate into CD1a+ mDC (mo-DC).[13] CD1a is one of the classical markers that is absent in all blood mDC and pDC subsets, and it is considered the specific marker for mo-DC.[14] Recently, it was reported that mo-DC do exist and differentiate *in vivo* in a *Leishmania* infection model.[19] This report provided for the first time evidence that *in vitro* differentiated mo-DC is not an *in vitro* artifact but reflect a population that is present *in vivo*.

We (Figure 2A) and others have demonstrated the presence of CD1a+ mDC in RA synovium.[20-24] Later, mature CD1a+ mDC were reported to be primarily located in the perivascular areas and ectopic lymphoid-like structures within the RA ST.[25] Altogether, a role for CD1a+ mDC in disease initiation and perpetuation has been proposed.

Interestingly, this DC subset has been associated with the presentation of human cartilage glycoprotein 39 (HCgp39, or YKL-40), an RA candidate autoantigen, both *in vivo*[26] and *in vitro*.[27,28] Presentation of the immunodominant epitope of HCgp39 by synovial DC, in the context of the shared epitope, was associated with characteristic histological features of follicular synovitis and was found to be highly specific for RA.[29]

CD1a+ mDC have also been reported to express high levels of Jak3, STAT4, and STAT6 in seropositive (rheumatoid factor positive) RA ST.[24] This study suggested that these markers might be alternative markers for DC in their early stages of activation, providing a tool for identifying RA at the level of the synovium. Moreover, the authors proposed that Jak3 inhibition might be a potential therapeutic target to prevent DC maturation in RA, thereby influencing T cell activation. Recently, the same group has extended their initial observations and reported that in RA ST Jak3+ DC were not confined to a single DC subset, with cells having phenotypes consistent with both myeloid- and plasmacytoid-type DC. Moreover, the activation status of these DC might suggest that DC were maturing or were fully matured,[30] which is in line with our own observations (MC Lebre *et al.* Am J Pathol. *in press*).

DC may also be involved in the

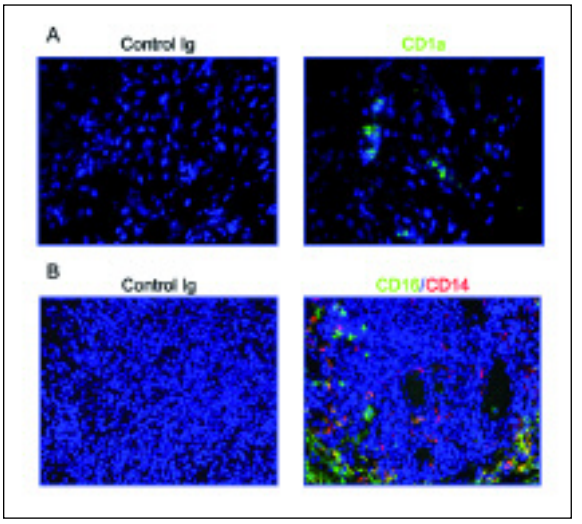

Figure 2. Expression of two mDC subsets in RA synovial tissue. Immunofluorescence staining of frozen sections from a representative RA ST using specific antibodies against CD1a (mDC) and double immnoflourescence staining using anti-CD14 and anti-CD16 antibodies. (A) $CD1a^+$ mDC stained green. (B) $CD14^+CD16^-$ monocytes stained red, $CD14^+CD16^+$ monocytes stained yellow/orange and probably the single CD16 positive cells are $CD16^+$ mDC. Original magnification 250x.

process of joint destruction in RA. The receptor activator of NF-kB (RANK)/RANK ligand (RANKL) pathway is critical in this process.[31] In this respect, it is of particular interest that in RA ST immature $CD1a^+$ mDC expressed both RANK and RANKL, while some mature DC-$LAMP^+$ DC expressed only RANK.[32] In this study, RANK expression appeared to be limited to the sites of inflammation (RA synovium). As RANK/RANKL interactions could be important for DC-T cell interactions during the inflammatory process, therapeutic control of these targets may have both positive and negative consequences for the immune system.

Several studies using *in vitro* generated RA mo-DC ($CD1a^+$ mDC) allowed more detailed investigations of the roles of these cells in RA. The first study demonstrated that in contrast to normal PB DC precursors, mo-DC derived from RA SF were resistant to the immunosuppressive effect of IL-10 *in vitro*.[33] This unresponsiveness might be regulated through modulation of cell surface IL-10R1 expression or signaling. These data imply that synovial $CD1a^+$ mDC maintain their inflammatory potential, even in the presence of an anti-inflammatory cytokine, contributing in this way to RA synovial inflammation. Interestingly, the existence of DC progenitors and mDC growth factors in RA SF supports the concept that RA SF may be a reservoir for joint-associated DC and reveals a compelling mechanism for the amplification and perpetuation of DC-driven responses in the RA joint, including inflammatory-type Th1 responses[34] and probably Th17 responses.

Moreover, RA SF inducible heat shock protein (ihsp)70 exhibited chaperoning potential, as indicated by the capture of ihsp70 present in RA SF in the surface of normal mo-DC. These data indicate that ihsp70 might chaperone autologous antigens into immature RA SF DC via hsp receptors (hspR), and that cross-talk between DC co-expressing hsp/hspR could reflect a disease process in RA.[35]

Several studies have investigated the role of Fcγ receptors (FcγR) in RA mo-DC. Mature mo-DC from RA patients showed a markedly increased production of IL-1, IL-6, TNF-α, and IL-10 compared with DC from healthy controls. When mo-DC (immature and mature) were triggered by FcγR the production of pro-inflammatory cytokines IL-1 and IL-12p70 decreased. Triggering of FcγR-independent mechanisms using IFN-γ increased the production of pro-inflammatory and Th1 cytokines, which was more pronounced in RA. FcγR dependent pathways influence cytokine production by DC.[36] These data suggest that a skewed balance towards pro-inflammatory and Th1 cytokines in RA can, at least partly, be restored by triggering FcγR on DC in RA and may lead to new strategies to abrogate Th1-driven inflammatory processes in RA.[36] In addition, immature DC from patients with active RA but not from patients with inactive RA or healthy controls markedly up-regulated FcγRII. Mature DC from patients with active RA also lacked the physiological down regulation of FcγRII that occurs upon maturation in control groups. FcγR-dependent stimulation of DC using antigen-IgG immune complexes (IC) significantly increased TNF-α production by DC from healthy controls, but significantly decreased TNF-α by RA DC. Overlapping expression patterns between FcγRII and DC-LAMP in the ST of patients with RA may imply that *in vivo*, mature DC express increased levels of FcγRIIb.[37] Moreover, RA mo-DC have increased expression of CCL18, CCL19 and CCL17 and this expression is partly regulated by FcγR triggering and results in an inhibitory DC subtype in RA upon FcγR-mediated triggering.[38] Of particular interest, RA mo-DC lacked the IL-13-

-mediated increase of FcγRII expression, with clear functional consequences. RA DC co-cultured with IL-4 already displayed an inhibitory DC phenotype, but this inhibitory phenotype was not augmented by the addition of IL-13. The defective FcγRII regulation was further substantiated by the finding that IL-13-generated DC from healthy controls increased antigen uptake capacity, whereas RA IL-13-generated DC did not. These data suggest that IL-13, by regulating the expression of FcγRII in normal subjects but not in RA, potentially contributes to the chronic pro-inflammatory immune reaction in RA.[39] Finally, it was reported that the functional variant of the inhibitory FcγRIIb (CD32B) is associated with the rate of radiological joint damage and DC function in RA.[40]

TLRs are involved in the regulation of DC activation and cytokine production.[41] In this respect, TLR2- and TLR4-mediated stimulation of mo-DC from RA patients resulted in markedly higher production of inflammatory mediators (TNF-α and IL-6) compared to DC from healthy controls.[42] The authors suggested that various TLR ligands in the joint may trigger multiple TLRs simultaneously, favoring the breakthrough of tolerance in RA.

Another cytokine that is increased by mo-DC in RA upon TLR triggering is macrophage migration inhibitory factor (MIF).[43] MIF is elevated in the serum of patients with RA to concentrations that are sufficient to induce leukocyte activation in vitro.[44] MIF production by DC may thus play a potential role in the amplification of the inflammatory loop.

Despite recent data indicating that mDC may be over-represented in RA, relatively little is known about the mechanisms promoting differentiation along specific DC pathways within distinct joint microenvironments.[34] Immature human mo-DC transdifferentiated into functional osteoclasts in the presence of M-CSF and RANKL. Importantly, this process was greatly enhanced by RA SF and involved pro-inflammatory cytokines such as IL-1 or TNF-α, as well as components of the extracellular matrix as hyaluronic acid.[45] DC-derived osteoclasts may thus represent key therapeutic targets in inflammation-induced bone resorption.

In addition, a unique natural killer (NK) cell subset ($CD3^-CD56^{bright}$) that accumulates in lymph nodes and chronically inflamed tissues triggered $CD14^+$ monocytes to differentiate into potent Th1-promoting DC. This process required direct contact of monocytes with NK cells and it was mediated by GM-CSF and CD154 derived from NK cells. It is noteworthy that RA SF, but not osteoarthritis SF, induced monocytes to differentiate into DC. However, this process occurred only in the presence of NK cells.[45] NK cells might play a role in the maintenance of Th1-mediated inflammatory diseases such as RA by providing a local milieu for monocytes to differentiate into DC.[46]

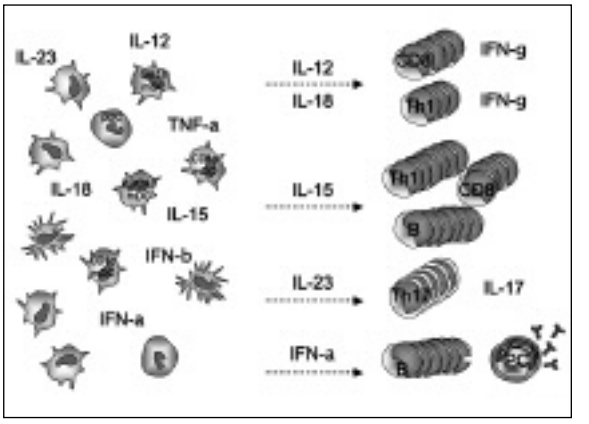

Figure 3. Schematic representation of the potential roles of DC subsets-derived cytokines in RA. In this hypothesis, synovial DC subsets might have the potential of perpetuating the inflammatory process seen in RA ST via the secretion of specific cytokines. In this respect, TNF-a, probably highly produced by $CD16^+$ mDC is a crucial pro-inflammatory and immunoregulatory cytokine that is central to the pathogenesis of RA will enhance synovial inflammation. IL-12 and IL-18 derived from $CD1c^+$ mDC might induce the release of IFN-a by memory CD4 and CD8 T cells. IL-18 release from pDC might induce the recruitment of more pDC into the synovial compartment in a positive loop. IL-15 released by mDC and pDC induces the proliferation of T and B lymphocytes. Importantly, IL-23p19 released by both mDC and pDC in RA ST might induce the expansion of Th17 cells. Finally, IFN-a released by pDC might induce the differentiation of B cells into (auto)antibody-producing plasma cells.

We have recently observed that RA synovial fibroblasts or their soluble factors present in conditioning medium are able to induce the generation of $CD1a^+$ mDC from peripheral blood monocytes (MC Lebre *et al.*, unpublished observations). Taking together, these data suggest an important role for the synovial microenvironment in the commitment of monocyte-derived cells and might support the generation of the synovial $CD1a^+$ mDC pool.

CD141(BDCA3)$^+$ mDC

To present no data on this peculiar DC subset in RA has been reported, except that CD141/

/BDCA3$^+$ mDC are present in RA ST (Figure 1, middle panel).

CD16$^+$ mDC

Randolph *et al.*[47] have demonstrated that CD16$^+$ monocytes are able to differentiate into DC *in vitro*, in the absence of exogenous cytokines, by using an experimental system of reverse transendothelial migration. So far, the existence of CD16$^+$ mDC in RA has not yet been reported, probably due to the lack of specific antibodies that characterized this DC subset. In this respect, the CD16 molecule is also expressed by human NK cells. NK cells are phenotypically defined as CD3$^-$CD56$^+$ lymphocytes. In healthy controls, CD56^{dim}CD16^{bright} cells represent at least 90% of all NK cells.[48] As the majority of the SF NK cells lack CD16 expression[49,50] is tempting to speculate that in RA ST CD16$^+$CD14$^-$ are CD16$^+$ mDC (Figure 2B, single positive cells seen in green).

Plasmacytoid DC in RA

CD123$^+$/CD303(BDCA2)$^+$/CD304(BDCA4)$^+$ pDC

Plasmacytoid DC constitute a rare population of immature DC, which lack myeloid markers, show plasmacytoid morphology, and differentiate *in vitro* into mature DC following stimulation with CD40L, viruses, and bacterial DNA.[51,52] Importantly, pDC have been shown to induce T -dependent[53] and -independent[54] B cell differentiation into antibody-producing plasma cells.

In 2004, Lande *et al.* reported for the first time the presence of pDC in RA.[55] In this study, the percentage of pDC, identified as a population of Lin$^-$CD123^{++} cells, was 4- to 5-fold higher in RA SF and psoriatic arthritis (PsA) SF compared with osteoarthritis SF. The morphological and immunophenotypic characterization of isolated pDC from RA SF showed that these cells were in an immature state, most likely due to inhibitory factors present in RA SF. However, pDC were still able to undergo maturation when exposed *ex-vivo* to viral agent or unmethylated DNA.[55] In addition, CD123$^+$ and BDCA2$^+$ pDC were detected by immunohistochemistry in RAST in which expression of the IFN-α-inducible protein MxA was also found, suggesting production of type I IFN by maturing pDC.

Differentiated DC and other APC are characterized by the nuclear location of RelB, a member of the nuclear factor (NF-)kB/Rel family.[56] Nuclear RelB$^-$CD123$^+$ pDC were located in perivascular regions of RA, with similar frequency compared to nuclear RelB$^+$CD123$^-$ mDC, but not normal ST sublining. This study also reported that the numbers and phenotypes of SF pDC were similar compared to those of normal PB pDC. In terms of antigen presentation capacity, PB pDC were less efficient than mDC. However, RA SF pDC efficiently activated resting allogeneic PB T cells.[57] Thus, pDCs are recruited to RA ST and comprise an APC population that might contribute significantly to the local inflammatory environment.

We have shown that circulating PB CD303/ /BDCA2$^+$ pDC (and also CD1c$^+$ mDC) numbers were significantly reduced in RA patients compared with healthy controls and displayed an immature phenotype with low CD62L expression. In RA SF pDC were present with the mDC:pDC ratio significantly exceeding that in matched peripheral blood. pDC isolated from RA SF also displayed an immature phenotype however, displayed a more mature phenotype (increased expression of CD80, CD83 and CD86) compared with PB mDC. Since pDC maturation is incomplete in the inflamed synovial compartment, immature pDC in SF may contribute to the perpetuation of inflammation via sampling of the inflamed synovial environment, and *in situ* presentation of arthritogenic antigen.[18] To extend these observations we have extensively analyzed the distribution and phenotype of pDC within, and between, RA, PsA and inflammatory OA synovia (MC Lebre *et al.* Am J Pathol. *in press*). CD304/BDCA4$^+$ pDC numbers exceeded CD1c$^+$ mDC only in RA, with the majority of infiltrating DC displaying an immature phenotype. Analysis of *in situ* cytokine expression revealed that pDC expressed IL-15, IL-18, IFN-α and IFN-β. In general, our results indicate that synovial DC (CD304/BDCA4$^+$ pDC and CD1c$^+$ mDC) might play an important role in synovial inflammation, in part via production of inflammatory cytokines. Importantly, type I IFNs released by pDC might also play a role in (auto-)antibody production by B cells. In line with this hypothesis is of particular interest that synovial pDC numbers were especially increased in RA patients who were positive anti-citrullinated peptide antibody (ACPA) and that synovial pDC numbers are positively correlated with the ACPA serum levels (MC Lebre *et al.* Am J Pathol. *in press*). Thus, immunomodulation by targeting synovial DC may provide a novel anti-rheumatic strategy.

Targeting DC in RA

RA is one of the most extensively studied diseases with regards to the tissue-specific attack of the joints leading to joint and bone destruction. In line with the studies on the potential roles of DC in RA, agents that inhibit DC differentiation and function might have therapeutic potential in the treatment of RA.

In this respect, the first report on the effect of therapy in RA DC showed that non-steroidal anti--oestrogens inhibited the differentiation of synovial macrophages into DC and the capacity of SF macrophage-derived DC to stimulate allogeneic T cells.[58]

The biologic agent anti-TNF has proven to be effective in treating patients with RA.[59] Analysis of the phenotype of circulating DC in RA patients before and after treatment with infliximab (at 24 h and 6 months) was recently undertaken and the correlation between these changes and the clinical response to treatment was assessed.[60] A decrease in circulating $CD11c^+$ mDC and, to a lesser extent, $CD123^+$ pDC percentages was found after infliximab therapy. The expression of CD83, the most important activation marker for DC, was also shown to be decreased 24 h after infliximab therapy. After 6 months of treatment, all patients showed significant clinical improvement and expression of the activation marker on DC remained low. In conclusion, this study supports the role for TNF-α blockade in preventing the maturation of circulating DC and in reducing the expression of their activation markers. Although the clinical response to infliximab was not observed after 24 h, circulating DC activation was strongly reduced by anti--TNF-α therapy. After 6 months of treatment, recent data showed a less active phenotype of DC associated with clinical improvement in all patients in the study. In line with these observations, *in vitro* studies using mo-DC demonstrated that the neutralization of TNF-α during the differentiation and maturation of DC did not result in an altered DC phenotype in RA or healthy controls. In contrast, the expression of CCL17, CCL18, CCL19, CCL22, CCL3, and CXCL8 by DC was significantly reduced when TNF-α activity was inhibited during LPS-triggered DC maturation. Moreover, the production of IL-1ß and IL-6 by mature DC was inhibited by PEGsTNFRI.[61] These data support at least partly the therapeutic effects of neutralizing TNF-α *in vivo*.

Another study investigated the effect of another TNF-α blocking agent, adalimumab, on DC after 12 weeks of therapy.[62] No significant differences in the numbers of circulating DC subsets ($CD11c^+$ mDC and $CD123^+$ pDC) were observed after adalimumab therapy.[62] Further functional and *in situ* studies are needed to unravel the role and distribution of more defined DC subsets after anti-TNF therapy.

DC in experimental arthritis

Data on the role of DC subsets in experimental arthritis are lacking. The only study that points to a role for DC in collagen-induced arthritis (CIA) in mice (the most common animal model for human RA used) was demonstrated by Leung *et al.*[63] These authors demonstrated that presentation of collagen-derived peptides by mature bone-marrow derived-DC is sufficient for the induction of arthritis in DBA/1 mice and that CIA could be inhibited by treatment with TNF antagonists. Importantly, CIA was antigen-specific, as transfer of control, unpulsed DC, or DC pulsed with ovalbumin did not induce arthritis. In contrast to other experimental arthritis models, DC-induced arthritis localized to the site of injection and did not spontaneously generalize to uninvolved joints, despite the demonstration of circulating collagen-reactive T cells. Histological analysis also revealed that DC induced arthritis (DCIA) exhibited extensive synovial hyperplasia and the appearance also resembled those of complete Freund's adjuvant-induced CIA and RA ST. Furthermore, collagen-pulsed DC were observed in the T cell areas of popliteal and inguinal lymph nodes and some of the $CD4^+$ T cells associated with these DC were activated. This supports the role for DC in the inductive phase of arthritis in this model, involving activation of T cells in local lymph nodes.

In a recent study with adjuvant-induced arthritis (AA) in rats, putative DC in normal rat synovial rich tissues (SRT) were characterized.[64] Flow cytometry showed that approximately 25% of the cells expressed CD45 and that approximately 60% of these $CD45^+$ cells expressed surface and/or cytoplasmic MHC class II molecules. Three subsets of putative APC were identified: $CD45^+MHCII^{hi}$, $CD45^+MHCII^{lo}$ and $CD45^+MHCII^-$. The $MHCII^{hi}$ cells had the phenotype $CD11c^+$, $CD163^-$ and $CD68^+$, similar to that of rat DC.[65] However, further

studies are required to define the precise cellular origins of DC and the factors responsible for the maturation of DC in rat inflammatory arthritis. T cell-induced inflammation in synovium is accompanied by an increase in mDC, macrophages and an incompletely characterized subset of MHC IIhiCD11b- non-lymphoid cells.[66] The presence of many MHC IIhi monocyte-like cells in inflamed SRT suggests that differentiation of monocytes is deviated towards DC, while the smaller proportion of "indeterminate" cells and the greater numbers of cells that expressed co-stimulatory molecules suggested that these cells will be different functionally from those in normal SRT.

Most of the present data on DC in experimental arthritis reported the use of DC as a therapeutic tool to ameliorate arthritis. In this respect, vaccination of mice with CIA with immature or LPS-activated DC had no significant effect on the disease course; administration of antigen-loaded, TNF--modulated DC propagated in GM-CSF with or without IL-4 resulted in a delayed onset of arthritis and a lower clinical score. The response was antigen-specific, since TNF-treated DC pulsed with a control antigen did not modify the disease course. A specific decrease in the collagen-specific «Th1-associated» IgG2a response was observed, whereas IgG1 titers were unaffected.[67] These findings provide a rationale for immunotherapy using DC in RA.

In addition, it was recently reported that repetitive immature DC injections triggered the expansion of a novel regulatory population with high immunomodulatory properties, able to protect mice from CIA. These regulatory T cells were characterized by the expression of the CD49b molecule and produced the anti-inflammatory cytokine IL-10.[68] Thus, together these data demonstrated that immature DC can expand and activate a novel regulatory population of $CD49b^+$ T cells, with high immunosuppressive potential able to mediate protection against a systemic autoimmune disease.

To gain a better understanding of the abilities and mechanisms by which immunomodulatory DC might influence the outcome of T cell responses, several immunomodulatory DC (TNF-, IL-10-, or dexamethasone-stimulated bone marrow-derived DC) were studied side by side for their ability to modulate T cell responses and autoimmune diseases. This report showed that differentially modulated DC display a different composition of molecules involved in T cell activation. Although all DC subsets analyzed were able to inhibit the induction of CIA in mice, the modulation of the underlying immune response was different. Vaccination with TNF- or IL-10-modulated DC altered the Th1/Th2 balance as evidenced by the induction of IL-5- and IL-10-secreting T cells and the concomitant reduction of the IgG2a-IgG1 ratio against the immunizing antigen. In contrast, DC modulated with dexamethasone did not affect the ratio of IL--5-producing versus IFN-α-producing T cells and tended to affect the antibody response in a non-specific manner.[69] These data indicate that distinct mechanisms can be used by distinct DC subsets to change the outcome of autoimmunity.

In another model of experimental arthritis, namely antigen-induced arthritis in C57BL/6 mice, it was shown that injection with antigen-(methylated BSA, mBSA)-exposed BAY 11-7082-treated DC resulted in suppressed inflammation and erosion, but not in those that received control antigen-exposed BAY 11-7082-treated DC. Clinical improvement was dependent on IL-10 and was associated with antigen-specific suppression of the delayed-type hypersensitivity reaction and switching of anti-mBSA antibody isotype from IgG2b to IgG1 and IgA. Suppression could be reversed by intra-articular administration of IL-1α and could be restored by a second injection of mBSA-exposed BAY 11-7082-treated DC.[70] In conclusion, BAY 11-7082-treated DC induced antigen-specific immune suppression in this model of inflammatory arthritis, even after full clinical expression of the disease. Such DC may have potential as antigen-specific therapy for autoimmune inflammatory arthritis, including RA.

Recently, the possibility of blocking antigen presentation of the type II collagen (CII)-derived immunodominant arthritogenic epitope CII259-273 to specific CD4 T cells by inhibition of antigen uptake in HLA-DR1-transgenic mice *in vitro* and *in vivo* was addressed. The authors showed that CII accumulated in membrane fractions of intermediate density corresponding to late endosomes. When DC and macrophages were treated with cytochalasin D or amiloride the intracellular appearance of CII was prevented and antigen presentation of CII259-273 to HLA-DR1-restricted T cell hybridomas was blocked. These data suggest that CII was taken up by DC and macrophages predominantly via macropinocytosis. Administration of amiloride *in vivo* prevented activation of CII-specific polyclonal T cells in the draining popliteal

lymph nodes.[71] This study suggests that selective targeting of CII internalization in professional APC prevents activation of autoimmune T cells, constituting a novel therapeutic strategy for the immunotherapy of RA.

Finally, a recent study investigated the potential of LF 15-0195 (LF), an NF-kb inhibiting agent, to generate immature tolerogenic DC that could be used for antigen-specific immunotherapy *in vivo* (in mice CIA).[72] Treatment of DC progenitors with LF resulted in a population of tolerogenic DC that was characterized by low expression of MHC class II, CD40, and CD86 molecules, as well as by poor allostimulatory capacity in a mixed leukocyte reaction. The efficacy of LF-treated DC in preventing arthritis was substantiated by histological examination, which revealed a significant decrease in inflammatory cell infiltration in the joints.[72] This study demonstrated that *in vitro*-generated antigen-specific immature DC may have important potential as a tolerogenic vaccine for the treatment of autoimmune arthritis.

Concluding remarks

As DC are APC which are key players in initiating specific immune responses by linking innate immunity with adaptive immunity, it is of particular interest to investigate the potential role(s) of these cells and design DC-based strategies to modulate the arthritic process. Thus, DC are of great interest for clinical applications in autoimmunity; a DC subset that can induce antigen/disease-specific tolerance would be highly desirable. Therefore, it is important to dissect the functional characteristics of DC subsets in arthritis in order to reveal whether (or which) DC subset is the problem, the solution or both.

Intense research of the immunobiology of RA has stimulated the introduction of novel approaches aimed at blocking inflammatory cytokine pathways in the synovial joint (e.g. TNF-α). However, an ever-increasing number of inflammatory molecules are being identified which contribute to RA pathology. As these inflammatory molecules have redundant effects on activating neighboring cells, therapies aimed at blocking multiple pathways may be required. Strategies aimed at identifying DC-derived molecules (that may represent ideal targets for gene therapy and systemic targeted therapies) involved in initiation/perpetuation of arthritis, T cell stimulation and B cell differentiation into plasma cell antibody production, hold great promise for future treatment of RA.

However, the functional roles of DC subsets in CIA and in RA remain to be established. Achieving a detailed understanding of the specific DC functions in RA holds potential for modulating DC for immunotherapy by down-regulating the autoimmune response.

Acknowledgements

The authors which to acknowledge Saïda Aarrass for excellent technical assistance and EULAR for providing financial support (EULAR Young Investigator Award 2005, to M.C.L.) and also the Dutch Arthritis Foundation (2008, nr. 0701014, to P.P.T. and M.C.L.)

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MULTIPLE FACTORS DETERMINE THE INCREASED PREVALENCE OF ATHEROSCLEROSIS IN RHEUMATOID ARTHRITIS

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Abstract

Rheumatoid arthritis (RA) is a systemic inflammatory disease that presents not only involvement of joints but also endothelial dysfunction, dyslipidemia, and premature atherosclerosis. The death rate in RA is known to be higher than in the general population and clinical cardiovascular events secondary to atherosclerosis are responsible for the excessive death rate. A better understanding of the mechanisms that take part in the pathogenesis of atherosclerosis in RA patients is needed. Thus, the authors review the role of several factors involved in RA atherosclerosis, including disease activity, new cardiovascular risk factors, dyslipidemia and the association of atherosclerosis with the use of anti-rheumatic drugs, glucocorticoids and anti-tumor necrosis factor (TNF) agents. The role of humoral autoimmunity, namely autoantibodies against heat shock proteins, cardiolipin and beta2--glycoprotein I, and its link with atherosclerosis is also discussed.

It is likely that the elucidation of the key mechanisms of atherogenesis in RA may determine a positive impact by reducing cardiovascular morbidity and mortality of these patients.

Keywords: Atherosclerosis; Rheumatoid Arthritis; Inflammation; Autoantibodies; Heat Shock Proteins.

Resumo

A Artrite Reumatóide (AR) é uma doença inflamatória sistémica que, para além do envolvimento articular, apresenta também disfunção endotelial, dislipidemia e aterosclerose prematura. A mortalidade na AR é superior à da população em geral e os eventos cardiovasculares secundários à aterosclerose são responsáveis por esse excesso de mortes. É necessário compreender melhor os mecanismos implicados na patogenia da aterosclerose nos doentes com AR. Os autores fazem uma revisão do papel de vários factores envolvidos na aterogénese na AR, incluindo a actividade da doença, novos factores de risco cardiovascular, a dislipidemia e a associação da aterosclerose com o uso de fármacos anti-reumáticos, glucocorticóides e agentes bloqueadores do factor de necrose tumoral (TNF). Também é discutido o papel da imunidade humoral, nomeadamente dos anticorpos contra proteínas de choque témico, cardiolipina e beta2-glicoproteina I, e a sua ligação à aterosclerose. É provável que a elucidação de mecanismos fulcrais da aterosclerose na AR possa ter um impacto positivo, traduzido na redução da morbilidade e mortalidade cardiovascular destes doentes.

Palavras-chave: Aterosclerose; Artrite Reumatóide; Inflamação; Proteinas de Choque Térmico; Auto--anticorpos

Introduction

Recently there has been a great advance in the diagnostic approach of rheumatoid arthritis (RA) after new laboratory tests, such as anti-cyclic citrullinated peptide (anti-CCP) antibodies, together with imaging methods, such as Ultrasound (US) and Magnetic Resonance of the affected joints, have allowed an early diagnosis and a better characterization of the initial clinical forms. These tests can also be predictive of the future severity of the disease.[1-10] In addition, there has been a recent paradigm shift in RA therapy, involving the concept of an early and aggressive treatment that has also contributed to a favourable change in the clinical evolution of these patients.[11-15] A better understanding of the immune pathogenesis has allowed the creation of new biological agents that act by blocking target

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cells and cytokines.[16-18]

Despite these new achievements, RA mortality rate is still higher than that of the general population and life expectancy remains reduced by 3 to 10 years.[19-21] Considering that cardiovascular problems are responsible for the higher mortality in RA, we reviewed important findings on the pathogenesis of atherosclerosis in this disease.

In severe RA, the increased mortality rate is comparable to that found in patients with lymphoma and triple vessel coronary artery disease.[22] The accelerated atherosclerosis determines a greater incidence of coronary artery disease in RA patients, which is responsible for the higher mortality rate when compared with the normal population.[23-31] There are countless studies on RA that confirm a higher frequency of carotid atherosclerosis, cerebrovascular ischemic events and coronary disease through several diagnostic methods such as carotid US, myocardium perfusion scintilography, and coronary artery angiography.[32-36] Atherosclerosis in the general population has been related to systemic inflammatory markers such as fibrinogen and, mainly, C reactive protein (CRP), which is consistent with the fact that atherosclerosis results from an inflammatory process in the artery and has even led some authors to suggest changing the nomenclature of atherosclerosis to atheroscleritis.[37-44] Currently, there are several studies in the population with coronary disease that search for new independent risk factors that could be predictive of atherosclerosis with the objective of increasing the early recognition of subclinical atherosclerosis, in an attempt to establish general preventive measures or even earlier therapeutic measures.[42,45-50]

Classical risk factors for atherosclerosis

In RA patients, the higher incidence of cardiovascular disease and the higher death rate result from the involvement of multiple pathogenic factors that contribute for the atherosclerotic lesion.[51,52] Some studies have depicted the occurrence of endothelial dysfunction, which is secondary to the diffuse vascular inflammation resulting from the disease activity.[53-56] Other studies have shown that RA patients present changes in the levels of lipoproteins with reduced levels of HDL cholesterol and high levels of total cholesterol, which are influenced by the disease activity and RA treatment.[57-61] The control of RA activity can improve this abnormal lipid profile and it was demonstrated that the use of non-steroidal anti-inflammatory drugs (NSAIDs), glucocorticoids and gold salts over a 9-month period increased significantly the HDL levels.[62] Moreover, the use of antimalarial agents has beneficial effects on the lipoprotein profile, with a reduction of the LDL cholesterol levels and an increase in the HDL cholesterol levels.[63,64]

Interestingly, the prevalence of the metabolic syndrome in Brazilian RA patients is 24%, and this finding is not significantly different from the general population.[65] In this study, there was no correlation between lipoprotein levels, glucose levels, hypertension, waist circumference, or body mass index (BMI) and disease activity parameters, functional capacity or response to therapy.[65]

Obesity is a classic risk factor for coronary atherosclerotic disease, however, a recent study in RA patients correlated BMI with cardiovascular mortality and showed that a low BMI (less than 20 kg/$/m^2$), usually associated with active RA, was in fact predictive of cardiovascular mortality, contrary to what is observed in the general population.[66]

There are few studies that define the role of other classic risk factors for cardiovascular disease in RA. The prevalence of diabetes mellitus (DM) is not higher in RA patients,[67] although there is a greater prevalence of insulin resistance, similar to what occurs in other systemic inflammatory conditions.[68,69] In fact, it is known that insulin resistance in RA is associated with disease activity and can improve with the use of glucocorticoids due to its anti-inflammatory effect, contrary to the findings found with this drug in non-RA patients.[70,71] On the other hand, smoking is an important risk factor for atherosclerosis in the general population, and tobacco has also been described as a major risk factor for RA onset.[72-74] The association between tobacco and RA is dose-dependent, that is to say, there is a correlation between tobacco consumption and disease severity and with the presence of rheumatoid factor (RF).[75-77] However, so far, published studies have not shown that tobacco is an independent risk factor for RA cardiovascular mortality. Thus classic risk factors for cardiovascular disease do not justify the greater incidence of accelerated atherosclerosis in RA patients.[59,78,79]

The role of some new risk factors for atherosclerosis, such as homocysteine and lipoprotein (a), have not been defined in RA, although preliminary results have already shown higher levels in these patients.[57,61,63,89-92]

The role of therapy and inflammation

RA severity markers have been associated with a greater global mortality, but no particular clinical parameters have been specifically related to cardiovascular mortality.[80] On the other hand, the relationship between atherosclerosis and RA treatment has also been analyzed and it was shown that the use of glucocorticoids and DMARDs in RA does not increase the incidence of cardiovascular disease, and some studies with methotrexate have even shown a reduction in cardiovascular mortality.[26,81-84] Most studies on the use of TNF blockers in RA patients have suggested beneficial effects on the endothelial function, although Van Doornum et al[85] described different results. Besides that, TNF blockers lead to improvement in insulin resistance[55,86,87] and reduce the risk of death and hospitalization secondary to cardiovascular disease.[88]

Recent studies have shown that a subgroup of RA patients have a greater number of T CD4+/ /CD28- lymphocytes cells, which produce gamma-interferon and induce the activation of Th1 cells with the resulting production of a variety of different proinflammatory cytokines. This observation is also described in atherosclerosis and it has been considered relevant in patients with unstable angina.[93] The T CD4+ CD28- cells, present in greater quantity in RA patients, are probably stimulated by endothelial autoantigens and infiltrate the atherosclerotic plaque, promoting vascular lesion due to their proinflammatory potential. A recent study confirmed the role of T CD4+ CD28- cells in the onset of early atherosclerosis in RA patients. In fact the authors showed that RA patients with a CD4+ CD28- expansion had increased intima-medial thickness (IMT) and greater endothelial dysfunction when compared with RA patients without expansion of those cells.[94]

It is considered that the inflammatory process in RA is, at least, partially mediated by T cells which may have as a result, not only joint inflammation but also the induction of inflammation on blood vessel walls. In addition, circulating RF and other immune complexes may also cause direct lesion of endothelial cells. Circulating proinflammatory cytokines released in RA may contribute to the inflammatory process on vessel walls, similar to what occurs in joints. On the other hand, TNFα and IL-6 induce the hepatic synthesis of CRP, which has been shown to be a prognostic factor for the development of cardiovascular disease secondary to atherosclerosis in the normal population. CRP induces the expression of adhesion molecules (ICAM 1, VCAM 1, and E-selectin) in endothelial cells, suggesting a direct pathogenic role for CRP in atherosclerosis.[95] Accordingly, in RA the systemic inflammation results in greater risk of cardiovascular mortality, and CRP levels were correlated with atherosclerosis in carotid arteries.[31,96] In addition, the increased cardiovascular mortality that occurs in RA patients is more frequent in patients with systemic involvement, such as rheumatoid lung disease and vasculitis, which could suggest the hypothesis of rheumatoid vasculitis as one of the triggering factors for atherosclerosis.[31] However, studies suggest that endothelial dysfunction, more than vasculitis itself is present in RA patients, and this dysfunction is independent of the patient age group, disease duration, disease activity, and RF levels.[97]

Brachial artery studies with high sensitivity US non-invasively assess endothelial function by measuring vasodilatation that occur after occlusion of the blood flow. In RA patients studies with this test have shown less artery vasodilatation and this was associated with disease activity. These findings suggest that chronic inflammation in RA is one of the responsible factors for the initial endothelial dysfunction, which starts the atherosclerotic process.[98,99] In RA the endothelial dysfunction is more intense in patients carrying the shared epitope.[100]

Coagulation parameters and atherosclerosis in RA

In the general population, studies show that fibrinolysis markers, such as fibrinogen, von Willebrand factor and the plasminogen activator inhibitor (PAI) were predictive of acute myocardial infarction.[101,102] These observations can be explained by the role of thrombosis caused by the unstable atherosclerotic plaque as an onset factor of acute coronary syndrome.[103] Systemic inflammation may be associated with a state of increased clotting due to thrombocytosis and high levels of fibrinogen, von Willebrand factor and PAI.[104] In RA patients the importance of these prothrombotic elements as predictors of a greater incidence of cardiovascular events secondary to accelerated atherogenesis has not been defined, due not only to the small number of studies, but also to the need of excluding patients with conditions that interfere with the mea-

surement of these parameters, such as DM and hormone replacement therapy. Studies show that high levels of fibrinogen, von Willerbrand factor, tissue plasminogen activator (tPA), fibrin D-dimer, and PAI were predictive of cardiovascular events in RA patients.[105-108]

Humoral autoimmunity and atherosclerosis of RA

Recent research in the general population suggests the participation of autoimmunity in atherosclerosis, with studies showing the association of atherosclerosis with antibodies against antigens expressed in the atheroma plaque, such as antibodies against oxidized LDL (LDLox), heat shock proteins (Hsp) 60 and Hsp 65, antibodies against membrane phospholipids and against beta2-glycoprotein I.[109-123] These antibodies are present more frequently in RA patients than in the normal population, but their pathogenic role in RA atherosclerosis requires further clarification.

Regarding antibodies against cardiolipin, their prevalence in RA is 15 to 20 percent, without association with any clinical manifestation of thrombosis; the association with clinical atherosclerosis has not been sought.[124,125] Additionally, studies have shown the induction of antibodies against phospholipids with the use of TNF blockers in RA patients, although the exact functional meaning of these antibodies in this context has not been clarified.[126]

In the general population, high levels of anti-LDLox have been associated with atherosclerosis and also with autoimmune rheumatic diseases such as systemic lupus erythematosus (SLE) and RA.[127-131] A recent study in RA showed a positive correlation between high levels of IgG autoantibodies against LDLox and carotid atherosclerosis, which suggests that autoimmunity present in RA is also a risk factor for atherosclerosis.[132] Finally, studies aiming to clarify the role of the proatherogenic proteins myeloperoxidase and PAPP-A in RA patients have not been carried out and could be important.

Carotid artery ultrasound in RA patients

Performing non-invasive studies such as US of the carotid arteries to recognize the presence of atherosclerosis in the preclinical phase and correlate these findings with clinical parameters is valuable, especially after the recent studies indicating that coronary disease in RA patients is more frequently silent, with a greater incidence of silent myocardial infarction and sudden death from cardiovascular causes.[133,134] In a 6.2 years follow up of 4476 non-RA adults who did not present evidence of cardiovascular disease, the increased IMT was associated with a greater incidence of AMI and cerebrovascular accident.[135] The presence of atherosclerotic plaques in carotid US predicts a higher risk of cardiovascular disease in the future and is considered an independent risk factor for AMI.[136-138] Many investigators have shown that RA patients present a greater mean carotid IMT when compared with the non-RA population.[33,34,132,139] The absence of standardized criteria to assess atherosclerosis in RA patients may explain some discordant results.[36,140] The increase of IMT in RA patients is correlated with disease duration and with inflammatory parameters such as CRP levels measured at the time of the US.[36] Roman *et al*, showed that the prevalence of subclinical atherosclerosis, defined by the presence of atheroma plaques in carotid artery US, is three times higher in a group of RA patients when compared to a control group paired by age, sex and ethnicity. The presence of plaques in this study did not correlate with the presence of hypertension, smoking, use of glucocorticoids, and low HDL level.[140] Two other studies also showed a greater carotid IMT in RA patients, however the presence of atheroma plaques was not increased.[32,33] In one of the studies, performed in patients from Korea, the low prevalence of plaques was suggested to be a consequence of the low prevalence of atherosclerosis and cardiac disease in that population.[33]

Conclusions

In summary, studies show that cardiovascular disease is a leading cause of mortality in RA and many factors contribute to this important clinical problem. Although classical risk factors such as smoking, dyslipidemia and others may be present in RA patients, they do not justify the higher prevalence of atherosclerotic disease. In fact, RA itself is an independent risk factor for coronary and cerebrovascular disease. In addition, the altered cellular immunity leads to endothelial lesions due to infiltration of the same inflammatory cells found in joints. The role of humoral immunity in the pathogene-

sis of atherosclerosis in RA patients is not yet well defined, since the few studies performed did not replicate the association with atherosclerosis that had been depicted in the general population.

It is important to consider that coronary artery disease in RA is often silent, and the extension of the lesions and event related mortality is higher when the diagnosis is only achieved after the clinical events have occurred. Therefore, the use of early atherosclerotic disease diagnostic tests, such as US, should be introduced more precociously in the evaluation of RA patients.

The associations found between disease activity and inflammatory parameters on one hand, and the decrease in mortality with the use of methotrexate and TNF blockers on the other hand, confirm the need for aggressive treatment in this disease, not only to prevent joint deformities but also to reduce cardiovascular events and the overall mortality.

Finally, rheumatoid synovitis shares some aspects with the inflammation that occurs in the atheroma plaque, meaning that RA can be viewed as an *in vivo* model of the complex inflammatory and autoimmune mechanism that occurs in atherosclerosis. Moreover, the evidence that in RA, intrinsic mechanisms initiate accelerated and early atherosclerosis can be used as a window of opportunity to improve our understanding of the initial mechanisms involved in cardiovascular diseases.

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HIPERHOMOCISTEINEMIA EM CRIANÇAS E ADOLESCENTES COM LÚPUS ERITEMATOSO SISTÊMICO: AVALIAÇÃO EVOLUTIVA

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Resumo

Objetivo: Um dos mecanismos implicados na patogênese da doença arterial coronariana dos pacientes com lúpus eritematoso sistêmico (LES) é a hiperhomocisteinemia. O nosso objetivo foi acompanhar evolutivamente pacientes com LES juvenil e detectar a presença e a persistência de hiperhomocisteinemia.
Pacientes e métodos: Foram avaliados dados demográficos e clínicos através dos prontuários de 18 pacientes com diagnóstico de LES juvenil (mediana de idade 13,5 anos). A homocisteína plasmática foi dosada em duas ocasiões com mediana de intervalo de tempo de 1,5 anos e associada com estado nutricional, atividade de doença, comprometimento renal e uso de metotrexato. A dosagem de homocisteína foi também realizada em 59 indivíduos saudáveis, pareados para idade e sexo.
Resultados: Dos 18 pacientes com LES, 16 (88,9%) eram do sexo feminino e 13 (72,2%) tinham comprometimento renal. Cinco (27,8%) dos 18 pacientes avaliados mantiveram concentração elevada de homocisteína nas duas dosagens (acima do percentil 90 do grupo de indivíduos saudáveis). A concentração aumentada de homocisteína não mostrou associação significante com presença de lesão renal (na primeira dosagem, p=0,676 e na segunda p=0,500), atividade de doença (na primeira dosagem, p=0,630 e na segunda p=0,182), sobrepeso/obesidade (na primeira dosagem, p=0,485 e na segunda, p=0,288) e nem com baixa estatura (na primeira dosagem, p=0,202 e na segunda, p=0,500).
Conclusão: Este estudo nos alerta para a persistência de concentrações elevadas de homocisteína plasmática em alguns pacientes com LES juvenil e para a necessidade de avaliações de intervenção terapêutica e orientação nutricional visando à redução de fatores de risco para doença cardiovascular.

Palavras-Chave: Lúpus Eritematoso Sistêmico; Homocisteína; Doença Cardiovascular; Crianças; Adolescentes.

Abstract

Introduction: One of the mechanisms implicated in the pathogenesis of coronary heart disease in patients with juvenile systemic lupus erythematosus (SLE) is the hyperhomocysteinemia. Our aim was to follow patients with juvenile SLE and to identify the presence and the persistence of hyperhomocysteinemia.
Methods: We studied 18 patients with juvenile SLE (median age 13.5 y). A survey of demographic and clinic data was performed based on patients records. The plasma homocysteine concentration was performed twice with a median interval of 1.5 years (1.3-2.5), and association with nutritional status, disease activity, renal involvement and use of methotrexate was sought. The plasma homocysteine concentration was also evaluated in 59 healthy controls, sex and age-matched to the patients.
Results: Of the 18 patients with juvenile SLE, 16 (88.9%) were female and 13 (72.2%) had renal involvement. Five out of 18 patients (27.8%) persisted with increased concentration of plasma homocysteine (above the 90th percentile of the healthy group). The elevated concentration of homocysteine did not show statistically significant association neither with renal involvement (in the first dosage, p=0.676 and in the second, p=0.500), disease activity (in the first dosage, p=0.630 and in the second, p=0.182), overweight/obesity (in the first dosage, p=0.485 and in the second, p=0.288) nor with short stature (in the first dosage, p=0.202 and in the se-

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cond, p=0.500).
Conclusion: This study emphasizes the persistence of elevated concentration of homocysteine in some patients with juvenile SLE and the need for evaluations of therapeutic strategies and nutritional education aiming to reduce risk factors of cardiovascular disease.

Keywords: Juvenile Systemic Lupus Erythematosus; Homocysteine; Cardiovascular Disease; Children; Adolescents.

Introdução

O lúpus eritematoso sistêmico é uma doença crônica, auto-imune caracterizada por inflamação generalizada de vasos sanguíneos e de tecido conjuntivo. Devido ao seu acometimento multissistêmico, suas manifestações clínicas são extremamente variáveis.

Nas últimas décadas tem havido um aumento na taxa de sobrevida dos pacientes com LES devido ao diagnóstico e ao tratamento precoces, ao uso de medicações mais eficazes e ao avanço de intervenções terapêuticas.[1] Como resultado do aumento da expectativa de vida, as crianças e adolescentes com LES têm apresentado considerável morbidade como seqüela de atividade de doença e da terapêutica, sendo a aterosclerose prematura um comprometimento importante.[2,3]

Diversos mecanismos parecem estar implicados na patogênese da doença arterial coronariana dos pacientes com LES, sendo esta provavelmente de origem multifatorial. A hiperhomocisteinemia é um desses fatores e ocorre em cerca de 15% dos pacientes adultos com LES.[4,5]

A homocisteína é um metabólito intermediário do grupo sulfidrila formado durante a conversão da metionina, que é um aminoácido proveniente da dieta. A deficiência de folato e de vitamina B12 prejudica a remetilação da homocisteína, que vai para o compartimento extracelular, incluindo o plasma. Isto explica porque a homocisteína colhida em jejum é um marcador da condição intracelular de folato e da vitamina B12.[6] Estudos experimentais sugerem que a hiperhomocisteinemia causa disfunção e lesão endotelial seguidas de ativação plaquetária e formação de trombos.[7,8] Trabalhos em adultos com LES têm evidenciado elevação da homocisteína relacionada com fenômenos tromboembólicos.[4,9,10]

Recentemente observamos, em dois estudos independentes, uma elevação de homocisteína plasmática em 47% e em 68,5% dos adolescentes com LES.[11,12] Não encontramos na literatura nenhum outro trabalho que tenha estudado a concentração de homocisteína em pacientes com LES juvenil. Estes achados associados à importância do aumento da homocisteína para o desenvolvimento de aterosclerose, em crianças e adolescentes com LES, nos motivou a acompanhar alguns pacientes evolutivamente com o objetivo de avaliar a presença ou persistência desta alteração e a possível correlação com a atividade clínica e presença de comprometimento renal.

Material e métodos

Pacientes

Em estudo anterior realizado em nosso serviço com 32 pacientes com LES, 18 tiveram uma segunda determinação de homocisteína plasmática em trabalho realizado posteriormente. Assim, foram selecionados para o presente estudo estas 18 crianças e adolescentes com diagnóstico de LES segundo os critérios de classificação do Colégio Americano de Reumatologia (ACR) que iniciaram a doença até os 18 anos de idade.[13] Todos os pacientes eram acompanhados em nosso serviço.

Os critérios de inclusão foram os seguintes: 1) preencher 4 ou mais critérios de classificação do ACR; 2) idade de início da doença superior a 10 anos e inferior a 18 anos; 3) pacientes com ou sem atividade clínica e laboratorial da doença.

Os critérios de exclusão foram: 1) presença de síndromes congênitas que alteram a homocisteína, 2) outras doenças associadas (diabetes, quadros renais não relacionados ao LES).

Foi realizado levantamento de dados demográficos, clínicos e laboratoriais e de tratamento com base nos prontuários.

Consideramos envolvimento renal se o paciente apresentasse classe III, IV ou V da OMS na biópsia renal, aumento nos níveis de creatinina ou alterações no sedimento urinário.[14]

O grau de atividade de doença (Systemic Lupus Erythematosus Disease Activity Index – SLEDAI) foi determinado em todos os pacientes no dia da coleta de sangue.[15]

O grupo de comparação incluiu 59 indivíduos saudáveis, pareados para sexo e idade com os pacientes e provenientes do mesmo meio sócio-econômico.

Nenhum paciente ou indivíduo saudável fazia uso de álcool ou tabaco.

De cada paciente e indivíduo saudável foi determinado o z escore do índice de massa corpórea (IMC) e o z escore de estatura para a idade.[16,17]

Os responsáveis pelos pacientes e indivíduos saudáveis assinaram o termo de consentimento informado para participar no estudo, que foi aprovado pelo Comitê de Ética em Pesquisa da Unifesp.

Métodos

Foram coletados de cada paciente com LES e de indivíduos saudáveis (pareados por idade e sexo com os pacientes), após jejum de 12 horas, 15 ml de sangue de veia periférica e colocados em tubo com anti-coagulante. Do plasma obtido, 500 µL foram armazenados a -80° Celsius e destinados à determinação da homocisteína. A concentração plasmática total de homocisteína foi feita através de cromatografia líquida de alto desempenho (HPLC) com detecção fluorimétrica e eluição isocrática.[18]

Para determinação das concentrações de normalidade da homocisteinemia, foi realizada a categorização pelo percentil 90 do grupo de indivíduos saudáveis (mediana de idade de 14,2 anos), e obteve-se o valor de 11,7 µmol/L, ou seja, foram considerados indivíduos com aumento da concentração de homocisteína aqueles com índice maior que 11,7 µmol/L. A dosagem de homocisteína dos pacientes foi realizada duas vezes com mediana de intervalo de tempo entre as duas dosagens de 1,5 anos (1,3 a 2,5 anos).

Estudo estatístico

Para análise estatística utilizou-se o programa SPSS 13.0. Os resultados são apresentados em tabelas de freqüência e de medidas de tendência central. Para avaliação das diferenças dos dados apresentados em forma categorizada utilizou-se o teste do Qui-quadrado. Para os dados apresentados de forma contínua utilizou-se o teste de Kolmogorov-Smirnof para testar a normalidade. Para avaliar as diferenças da mediana entre os grupos com LES e o de indivíduos saudáveis utilizou-se o teste de Mann-Whitney; e para a comparação dos valores de homocisteína nas duas dosagens, o teste pareado de Wilcoxon. Adotou-se a $< 0,05$.

Resultados

A mediana do tempo de evolução da doença foi de 15 meses (3 a 75 meses).

A mediana de idade dos pacientes avaliados, na primeira dosagem foi de 13,5 (11,6 a 16,7 anos) e na segunda dosagem de 15,5 (13,1 a 17,9 anos) (Quadro I). Dos 18 pacientes com LES 16 (88,9%) eram do sexo feminino, 14 (77,8%) caucasianos, 13 (72,2%) tinham comprometimento renal e 3 (16,7%) utilizavam metotrexato. Nenhum paciente apresentava doença vascular aterosclerótica ou trombótica prévia.

Quanto à condição nutricional 4 (22,2%) tinham sobrepeso/obesidade e 3 (16,7%) tinham baixa estatura, sem diferença estatisticamente significante entre a primeira e a segunda dosagens (Quadros I e II).

Não houve diferença estatisticamente significante entre o grupo de pacientes com LES e o grupo de indivíduos saudáveis em relação ao sexo, idade, raça e condição nutricional (Quadro I).

Cinco (27,8%) dos 18 pacientes avaliados mantiveram concentração elevada de homocisteína nas 2 dosagens, 4 (22,2%) apresentaram homocisteína elevada somente na primeira dosagem, 6 (33,3%) tiveram homocisteína elevada somente na segunda dosagem e 3 (16,7%) não tiveram elevação da homocisteína em nenhuma dosagem (Figura 1).

Em relação ao índice de atividade de doença (SLEDAI), observou-se que na primeira dosagem os pacientes estavam mais ativos do que na segunda, com diferença estatisticamente significante ($p = 0,030$). Entretanto, a concentração de homo-

Quadro I. Dados demográficos e nutricionais de pacientes com LES (N=18) e de indivíduos saudáveis (N=59)

Parâmetro	LES	Saudáveis	Valor de p
Idade	13,5 (11,6;16,7)	14,2 (10,0;18,0)	0,356
Sexo feminino	16 (88,9%)	52 (88,1%)	0,930
Raça caucasiana	14 (77,8%)	38 (64,4%)	0,288
IMC > p85	4 (22,2%)	12 (20,3%)	0,837
ZEI < -2	3 (16,7%)	3 (5,1%)	0,136

IMC: índice de massa corpórea
ZEI: z escore estatura/idade

Quadro II. Dados antropométricos, SLEDAI e nível de homocisteína nas duas dosagens de pacientes com LES (N=18)

Parâmetro	1ª Dosagem	2ª Dosagem	p
Índice de massa corporal > p85	4 (22,2%)	4 (22,2%)	0,472
z escore do IMC	0,67 (-0,86 a 2,07)	0,19 (-1,61 a 2,20)	0,472
z escore estatura/idade <-2	3 (16,7%)	2 (11,1%)	0,214
Z escore estatura/idade	-0,86 (-4,15 a 1,28)	-0,36 (-3,35 a 0,55)	0,214
SLEDAI	4,5 (0,0 a 23,0)	1,0 (0,0 a 14,0)	0,030*
Homocisteína > 11,7 µmol/L	11 (61,1%)	9 (50%)	0,432
Homocisteína	13,28 (8,25 a 22,37)	11,88 (9,10 a 21,10)	0,811

Wilcoxon Signed Ranks Test
* p < 0,05
IMC- Índice de Massa Corpórea

cisteína não diferiu estatisticamente nas duas avaliações. Em relação às outras variáveis estudadas não se observou diferença significativa (z escore do IMC, p= 0,472 e z escore de estatura para a idade, p = 0,214) (Quadro II).

A concentração aumentada de homocisteína não mostrou associação estatisticamente significante com a presença de comprometimento renal (na primeira dosagem, p = 0,676 e na segunda, p = 0,500), sobrepeso/obesidade (na primeira dosagem, p = 0,485 e na segunda, p = 0,288) e nem com a baixa estatura (na primeira dosagem, p = 0,202 e na segunda, p = 0,500). Não observamos associação entre o uso de metotrexato e concentrações séricas elevadas de homocisteína. Entretanto, devemos considerar que apenas 3 pacientes estavam fazendo uso desta medicação.

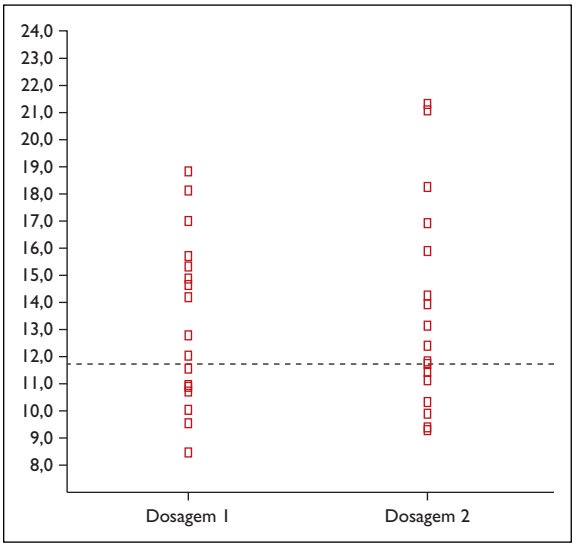

Figura 1. Valores de homocisteína dos pacientes avaliados nas duas dosagens (N=18)

Discussão

Embora não esteja estabelecido o papel exato da homocisteína no risco para a doença arterial coronariana, parece que ela atua através de efeitos tóxicos no endotélio vascular e através da alteração da atividade anti-coagulante.[5,7,19]

Estudos da literatura têm evidenciado que o aumento da concentração plasmática de homocisteína é um fator de risco independente para a doença cardiovascular, e uma importante causa de morbi/mortalidade nos pacientes com LES. Graham *et al*, em estudo multicêntrico europeu concluíram que a elevação da homocisteína em adultos aumenta em 2 vezes o risco de doença vascular em pacientes com alguma patologia vascular (cardíaca, neurológica ou periférica).[20]

Trabalhos em adultos com LES têm evidenciado elevação da homocisteína relacionada com fenômenos tromboembólicos.[4,9,10]

Em estudo transversal recente realizado com 32 crianças e adolescentes com LES, encontramos uma prevalência de 47% de elevação de homocisteína.[11] Aproximadamente 2 anos após, um outro estudo avaliou 35 pacientes com LES juvenil sendo que 18 destes tinham sido avaliados no estudo anterior.[12]

Não encontramos na literatura nenhum outro trabalho que tenha estudado a concentração de homocisteína em pacientes com LES juvenil. Também não existe estudo populacional em nosso meio em relação à concentração de homocisteína em indivíduos saudáveis, o que limita a compara-

ção dos nossos resultados. Fatores não avaliados por nós como risco familiar, estilo de vida e estado nutricional relacionado às vitaminas do complexo B e ao ácido fólico, envolvidos no metabolismo da homocisteína poderiam, eventualmente, explicar os nossos resultados. Sabe-se que deficiência destas vitaminas pode interferir na concentração plasmática de homocisteína.

Embora não tenhamos encontrado associação entre a concentração de homocisteína e a atividade da doença, não afastamos a possibilidade da influência desta associada a outros fatores, como o comprometimento renal e o uso de certas medicações.

Os possíveis mecanismos que levariam ao aumento da homocisteína em pacientes com alteração renal incluem a diminuição da excreção renal de homocisteína, o prejuízo no metabolismo renal, a inibição do metabolismo extra-renal de homocisteína pelas toxinas urêmicas ou a redução da vitamina B na falência renal.[21,22] Apesar de não termos encontrado associação estatística entre a presença de envolvimento renal e a elevação da homocisteína no presente trabalho, temos de levar em conta o tamanho da amostra e o fato de que medidas disponíveis para avaliação da função renal não são muito sensíveis.

Estudo realizado em adultos com artrite reumatóide mostrou correlação entre o uso do metotrexato e o aumento da concentração de homocisteína.[23] Entretanto, quando suplementados com ácido fólico, esses pacientes tiveram redução da concentração plasmática de homocisteína.[23] Em nosso estudo apenas três pacientes faziam uso da associação metotrexato e ácido fólico e nenhum apresentou elevação da homocisteína, provavelmente pelo uso concomitante desta vitamina.

Observamos que cerca de 28% dos pacientes mantiveram concentração elevada da homocisteína nas duas dosagens o que nos alerta para a importância de estudos de intervenção terapêutica e orientação nutricional para estes pacientes, visando à redução de fatores de risco para doença cardiovascular.

O nosso estudo foi transversal e não permitiu estabelecer relação entre hiperhomocisteinemia e o desenvolvimento de fenômenos tromboembólicos. Estudos de coorte com maior tempo de evolução poderão elucidar melhor este risco.

A freqüência de hiperhomocisteinemia é bastante elevada em crianças e adolescentes com LES. Embora na maioria dos pacientes a elevação da concentração de homocisteína seja transitória, em alguns ela pode persistir por tempo mais prolongado e é para estes pacientes que se devem voltar os nossos esforços no sentido de controlar este fator de risco e possivelmente fenômenos tromboembólicos futuros. Entretanto, a indicação da dosagem de homocisteína plasmática de rotina para avaliação do risco cardiovascular ainda é objeto de discussão.

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EULAR 2008

França, Paris
11-14 de Junho de 2008

XV Congresso de la Liga Panamericana de Asociaciones de Reumatologia

PANLAR, Guatemala
13-16 de Agosto de 2008

CLINICAL FEATURES OF JUVENILE SYSTEMIC LUPUS ERYTHEMATOSUS IN IRANIAN CHILDREN

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Abstract

Objective: Analysis of the clinical and laboratory features of childhood onset systemic lupus erythematosus (JSLE).

Patients and method: Forty five children, aged below 16, were enrolled in this retrospective multicenter study. All patients fulfilled the American College of Rheumatology revised criteria 1982 for the diagnosis of SLE and had shown clinical manifestations of the disease before the age of 16.

Results: The female to male ratio was 8:1. The mean age at onset was 10.5 (ranged between 3 and 16 years). Thirty patients (66%) were correctly diagnosed before referring to our Center.

The clinical manifestation in different organs were as follows: 40 patients (88.8%) had skin involvement, 35 patients (77.7%) experienced musculoskeletal involvement, 29 children (64.4%) suffered from renal disease, hematological abnormalities were detected in 25 patients (55.5%), 12 patients (26%) had cardiovascular disease, 10 patients (17%) presented central nervous system involvement, and 5 patients (11%) experienced SLE-related pulmonary disease. During the follow up period four patients died, two from renal failure, one from CNS complications of JSLE, and one due to severe sepsis.

Conclusion: Clinical manifestations of Juvenile SLE are diverse and often severe. Similar studies should be undertaken in different geographic areas in order to provide a good insight of the disease towards a correct diagnosis of JSLE.

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Keywords: Juvenile Systemic Lupus Erythematosus; JSLE; Clinical Features; Iran.

Resumo

Objectivo: Análise das características clínicas e laboratoriais do Lúpus Eritematoso Sistémico de início Juvenil (LESJ).

Doentes e métodos: Quarenta e cinco crianças de idade inferior a 16 anos foram incluídas neste estudo retrospectivo, multicêntrico. Todos os doentes preenchiam os critérios revistos do American College of Rheumatology de 1982 para o diagnóstico de LES e apresentaram as primeiras manifestações clínicas antes da idade de 16 anos.

Resultados: A relação sexo feminino/sexo masculino foi de 8:1. A idade média no início da doença foi de 10,5 anos (entre 3 e 16 anos). Trinta doentes (66%) foram correctamente diagnosticados antes da referenciação ao nosso Centro. As manifestações clínicas dos diversos órgãos foram as seguintes: 40 doentes (88,8%) tiveram envolvimento cutâneo, 35 (77,7%) atingimento músculoesquelético, 29 crianças (64,4%) apresentaram doença renal, alterações hematológicas foram detectadas em 25 (55,5%), 12 (26%) tiveram doença cardiovascular, 10 doentes (17%) apresentaram envolvimento do sistema nervoso central e 5 (11%) doença pulmonar relacionada com o LES. Durante o período de *follow up*, 4 doentes faleceram, dois em insuficiência renal, um por envolvimento do SNC e um por sépsis grave.

Conclusão: As manifestações clínicas do LES Juvenil são variadas e frequentemente graves. Estudos semelhantes devem ser realizados em diferentes áreas geográficas de forma a providenciar uma boa visão da doença para o correcto diagnóstico do LESJ.

Palavras-chave: Lúpus Eritematoso Sistémico Juvenil; LESJ; Manifestações Clínicas; Irão.

Introduction

Systemic Lupus Erythematosus (SLE) is a chronic autoimmune disease with a wide spectrum of clinical and immunological abnormalities.[1] SLE is primarily a disease of young adult women; however, in 10-15% of patients, the diagnosis is first established during childhood.[2,3] According to previous reports, Juvenile Systemic Lupus Erythematosus (JSLE) is rarely seen in children under 5 years of age and the peak incidence of childhood SLE occurs around puberty.[4,5]

The clinical manifestations of the disease, which are remarkably diverse, include fever, erythematous rash, polyarthralgia and arthritis, polyserositis, anemia, thrombocytopenia, renal, neurological and cardiac abnormalities such as pericarditis, myocarditis and endocarditis.[6] The importance of JSLE derives from the fact that it is a life-threatening, long-term illness associated with significant complications.[7] Moreover, the atypical presentation, common in this age group, is often responsible for major diagnostic delay.[3] In patients with childhood-onset SLE, the initial symptoms have been reported to be more severe than in adults.[3] Most studies of JSLE affecting children below the age of 16, have been reported from centers in North America or Europe.[8]

Taking into consideration the different clinical and epidemiological presentations in various ethnic groups, the aim of our study was to review the clinical and laboratory features of 45 Iranian children with SLE.

Patients and Methods

We conducted a retrospective chart review study from May 1996 up to April 2006 in order to describe clinical manifestations and laboratory features among Iranian children with JSLE. The study protocol was approved by the Ethics Committee of the faculty.

The inclusion criteria were as follows:

1. Age ≤16 at diagnosis;
2. Fulfilling the revised American College of Rheumatology (ACR) 1982 criteria[1] for the diagnosis of SLE ;
3. Absence of drug induced SLE.

Children with systemic juvenile idiopathic arthritis, polymyositis and vasculitis were excluded from the study.

Standardized Questionnaires were designed and sent to three university-affiliated pediatric centers in Tehran. Data on clinical manifestations and laboratory findings, gender, date of birth, ethnic group, area of referral, and age at diagnosis were obtained by reviewing medical records. Detailed information of clinical disease expression was reviewed. This included involvement of skin, musculoskeletal, renal, pulmonary, cardiovascular, central nervous system, and hematological abnormalities. Laboratory investigations included complete blood count (CBC), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), kidney function tests, Coombs' test, VDRL, antinuclear antibodies (ANA), anti-double-stranded DNA (anti-dsDNA), anti-Smith antibody (anti-Sm), antiphospholipid antibodies and complement fractions (CH50, C3 and C4). The charts were reviewed by a pediatric rheumatologist and overall 45 patients were eligible to participate in our study.

Results

A sample of 45 patients with JSLE, aged between 3 and 16 years at the diagnosis of disease was studied. Forty patients (88.9%) were female and 5 (11.1%) were male (F to M ratio 8:1). Thirty five patients (77.7%) were from North-West of Iran (Kurdistan, and Azerbaijan) and the rest (22%) were from other ethnicities.

The mean age at disease onset was 10.5 ± 2.5 years. The majority of our study group had the diagnosis made within a few months of presentation and only 13% of patients had a delay in diagnosis up to five years. Most of the patients were referred to our centers within a few months of diagnosis, with an average of 3.5 months.

JSLE was correctly diagnosed at onset of the disease in 30 out of 45 patients (66.6%), whereas, the true diagnosis was established with delay in the other patients: leukemia and lymphoma, systemic onset juvenile idiopathic arthritis (SOJIA), immune thrombocytopenic purpura, and rheumatic fever, were among incorrect diagnosis initially made in the early stages of the disease in the rest of our patients.

Constitutional manifestation including fever and weight loss, were present in 43 patients (95%) with JSLE (Table I). The most common feature of the disease at onset was mucocutaneous involvement which affected 40 out of 45 patients (88.8%).

Table I. Clinical presentations of JSLE

	No	%
Constitutional	43/45	95%
Mucocutaneous	40/45	88.8%
Musculoskletal	35/45	77.7%
Renal	29/45	64.4%
Cardiovascular	12/45	26.6%
Central Nervous System	8/45	17%
Respiratory system	5/45	11.1%

Table II. Mucocutaneous manifestation

Cutaneous manifestation	**40 (45)**	**88.8%**
Butterfly Rash	30 (40)	75%
Alopecia	21 (40)	52%
Photosensitivity	15 (40)	37%
Non specific generalized Rash	10 (40)	25%
Discoid lesion	9 (40)	25%
Cutaneous Vasculitis	8 (40)	20%
Mucosal ulcer	8 (45)	17%

Table III. Musculoskeletal Manifestation (During the disease course)

Musculoskeletal manifestation	**N° 35/45**	**% 77.7%**
Arthritis	35/45	77.7%
Arthralgia	35/45	77.7%
Muscle weakness and myositis	5/45	11%

Table IV. Kidney involvement

Kidney involvement	**N° 29/45**	**% 64,4%**
Hematuria	25/29	86.2%
Proteinuria	22/29	75.8%
Raised BUN & Creatinine	15/29	51.7%
Renal biopsy	26/29	89.6%
Stage class II	4/26	15.3%
Stage class III	5/26	19.21%
Stage class IV	15/26	57.6%
Stage class V	2/26	7.6%

The frequency of various cutaneous manifestations is shown in Table II.

Musculoskeletal involvement was documented in 35 (77.7%) children. Arthritis in our patients was a non-deforming, self limited polyarthritis. Arthritis and arthralgia affected 35 (77.7%) patients. Muscle weakness and myositis was present only in 5 (11.1%) children (Table III).

Twenty nine children (64.4%) experienced renal involvement during the disease course. Hematuria and proteinuria were present in 25 (55.5%) and 22 (48.8%) patients respectively. BUN and Cr increased in 15 (33.3%) of our patients and 26 (57%) underwent renal biopsy. The results are summarized in Table IV.

Cardiovascular problems occurred in 12 patients (26.6%). Pericarditis and myocarditis, affected 7 (58.3%) and 4 (33.3%) children, respectively. Libmann-Sacks endocarditis was quite rare; it was seen in 2 (16.6%) patients during the disease course (Table V).

CNS involvement (documented in 8 patients, 17%) was manifested by non-organic brain disease and psychosis, each in 6 patients (13.3%) and seizure in 5 (11.1%) children; respiratory manifestations were present in 5 (11.1%) patients (Table V).

Anemia was the most common hematological abnormality in our patients (25 out of 45 patients), of which 15 were Coombs' negative and 10 Coombs' positive (Table VI). Leucopenia and thrombocytopenia were present in 8 (17%) and 6 (13%) patients respectively. Overall, 16 patients (35%) were Coombs' positive and 12 patients (26%) were VDRL positive (Table VI). ANA was detected in 43 patients (96%). The results of anti-dsDNA, antiphospholipid antibody, anti-Sm antibody and complement activity are summarized in Table VI.

Conclusion

This is the first report of JSLE from Iran, describing the course of 45 Iranian children with this relatively rare connective tissue disease. According to previous studies, the clinical manifestations of JSLE are similar to those of adults, but with more severe multiorgan involvement.[3, 9]

As mentioned, almost 78% of our patients were from North-West of Iran (Kurdistan, and Azerbaijan). This could be due to genetics predisposing factors and HLA associations in this part of Iran.

Mucocutaneous involvement was found in almost 89% of our patients at disease onset. Compared to series reported from Europe and the Middle

Table V. Other organs involvement

Organ involvement	Nº	Percent
Cardiovascular system	12/45	26%
Pericarditis	7/12	58.3%
Myocarditis	4/12	33.3%
Libmann-Sacks Endocarditis	2/12	16.6%
Central nervous system	8/45	17%
Non organic brain disease	6/45	13.3%
Psychosis	6/45	13.3%
Seizure	5/45	11.13%
Respiratory system	5/45	11.1%

Table VI. Laboratory findings in 45 Cases of JSLE

Lab test	Nº	Percent
Anemia Hb<8 gr/dl	25/45	55%
Coomb's positive anemia	10/25	40%
Coomb's negative anemia	15/25	60%
Leukopenia	8/45	17%
Thrombocytopenia (ITP)	6/45	13/3%
ESR> 85 mm/h	42/45	93%
CRP +++	40/45	88%
False positive VDRL	12/45	26%
Coomb's test Positive	16/45	35%
BUN > 40 mg/dl	13/45	33%
Hematuria	25/45	55%
Proteinuria	22/45	48%
ANA	43/45	96%
Anti- dsDNA	40/45	91%
Antiphospholipid antibodies	10/45	22%
Anti -Sm	5/45	13%
Low C3	38/45	85%
Low C4	18/45	41%
Low CH50	38/45	85%

East[3,10], mucocutaneous involvement at onset of JSLE seems to be more frequent in Iranian children what may be due to problems with low education and hygiene.

Arthritis and arthralgia were seen in 78% of our patients. This figure is less than that of Egyptian children (100%).[11] However, in comparison with France, Saudi Arabia, and United States of America our children more frequently experienced joint involvement. According to published reports, the manifestations of muscle involvement can range from generalized muscle ache in 40–80% of patients to frank inflammatory myositis in 5–11% of cases.[12] The histological appearance of muscle in patients with myositis secondary to lupus may be identical to that of patients with polymyositis.[12] Muscle weakness and myositis was noted in five cases (11%), which was almost similar to mentioned report.

Clinically significant renal involvement in systemic lupus erythematosus is more common in children than in adults.[7,13,14] At the time of diagnosis of SLE, 75% of children may be found to have renal involvement ranging from minor findings on urinalysis to significantly decreased renal function.[7] Almost 65% of our patients presented renal involvement at onset of the disease, similar to reports from Saudi Arabia and France.[3,10] Fifty seven percent of our patients underwent renal biopsy. Overall, 15 out of 45 (33.3%) had stage IV WHO renal involvement and 2 out of 45 (4.4%) had stage V WHO renal disease. Lupus can involve all parts of the heart. Twenty six out of 45 children with JSLE presented cardiac involvement at onset of the disease. Our patients were more likely to develop cardiac disease in comparison to French children[3], however, this figure seems to be lower compared to other reports.[10,11]

Pulmonary involvement has become increasingly recognized as a manifestation of SLE. Although it usually runs a benign course, pulmonary lupus sometimes carries a serious prognosis.[6] 11.1% of our patients experienced pulmonary disease. This problem is less frequent in our patients compared to Saudi Arabians and French children.

Significant neuropsychiatric (NP) is seen in SLE,[15] most presented as severe non organic manifestations. Neuropsychiatric complications occurred in 50% of JSLE patients.[16,17] The incidence of CNS involvement in our series is similar to that of reported in France, but significantly less than that of other reports.[10,11] The lack of systematic neuropsychological evaluation might be the cause of this low frequency of subtle CNS changes. However, compared to another report, we observed higher incidence of psychosis and seizure in our patients.[1]

The majority of our patients suffered from anemia (55%), 40% Coombs' positive comparable to other series. We observed significantly lower incidence of leucopenia in our children in comparison with other study groups.[3,10]

The mean titer for ANA was between 1:1280 and 1:640. ANA and anti-ds-DNA antibodies were de-

tected in 96% and 91% of our patients, respectively. This is comparable to what is reported in Saudi Arabia, but our patients were more frequently found to have anti ds-DNA antibody compared to reports from United States. Hypocomplementemia strongly suggests the diagnosis of lupus and identifies patients at increased risk for glomerulonephritis.[18,19] Our results were similar to those reported from France with regard to low level of complement.[3] We observed significantly lower incidence of anti-Sm antibody in our patients compared to children of other races.[1,3] The high positivity of ANA and anti ds-DNA , in addition to low complement level, at presentation of the disease suggest that these are helpful tests in diagnosing children suspected to have JSLE.

In conclusion, clinical manifestations of JSLE obviously differ in different races and ethnic groups. It is strongly recommended that similar studies be undertaken in different geographic areas in order to provide a good insight towards correct diagnosis of JSLE.

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AMENORREIA INDUZIDA POR CICLOFOSFAMIDA EM DOENTES PRÉ-MENOPÁUSICAS COM LÚPUS ERITEMATOSO SISTÉMICO

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Resumo

Objectivos: Avaliar a ocorrência de insuficiência ovárica em doentes lúpicas pré-menopáusicas tratadas com ciclofosfamida; identificar factores de risco para esta complicação; apreciar a ocorrência e viabilidade da gravidez durante e após o tratamento.

Métodos: Estudo retrospectivo de doentes submetidas a terapêutica endovenosa com ciclofosfamida no Serviço de Reumatologia dos Hospitais da Universidade de Coimbra, actualizado por entrevista. Foram recolhidas informações referentes a dados demográficos; história ginecológica e obstétrica; caracterização da doença; duração da terapêutica e efeitos secundários associados.

Insuficiência ovárica (IO) foi definida como ausência de menstruação por período igual ou superior a 4 meses e o diagnóstico foi confirmado por doseamentos hormonais.

Resultados: Foram submetidas a ciclofosfamida endovenosa no nosso Serviço, 19 mulheres pré-menopáusicas com lúpus eritematoso sistémico (LES), com uma média de idades ao início do tratamento de 28,4 anos. A glomerulonefrite lúpica, de diversas classes, foi a indicação principal para a terapêutica (89,5%). As doentes receberam, em média, 9,3 pulsos endovenosos, ao longo de 16,8 meses, numa dose cumulativa média de 6.973 mg. Três doentes apresentaram insuficiência ovárica (15,8%). Estas doentes tinham idade mais avançada ($P = 0,0016$). Uma doente engravidou durante o período de tratamento. Duas doentes tiveram filhos saudáveis após o tratamento.

Conclusões: Observámos IO em 15,8% das doentes tratadas. A idade no início da terapêutica com ciclofosfamida parece ser factor determinante, à semelhança do descrito na literatura. A gravidez durante o tratamento pode ocorrer, pelo que é mandatório garantir contracepção eficaz. Após ciclofosfamida, a gravidez é possível e com prognóstico favorável.

Palavras-Chave: Lúpus Eritematoso Sistémico; Insuficiência Ovárica; Ciclofosfamida; Gravidez.

*Serviço de Reumatologia dos Hospitais da Universidade de Coimbra

Abstract:

Objectives: To determine the frequency of ovarian failure in pre-menopausal women after cyclophosphamide (cyc) treatment for systemic lupus erythematosus (SLE); identify risk factors for this complication; estimate the occurrence and viability of pregnancy during and after treatment.

Methods: Review of the data of women treated with intravenous cyc in the department of Rheumatology of Hospitais da Universidade de Coimbra, up dated by interview. Information on demographic features; gynaecologic and obstetrical history; characteristics of the disease; duration and side effects of treatment were obtained. Ovarian failure was defined as a lack of menses for, at least, four months and the diagnosis was confirmed by hormonal measurements.

Results: Nineteen pre-menopausal women were treated with intravenous cyc in our department. The mean age at the time of cyc initiation was 28.4 years. Lupus nephritis was the most common indication for cyc treatment (89.5%). The mean number of pulses was 9.3 over a period of 16.8 months. The mean cumulative dosage was 6.973 mg. Three patients developed ovarian failure. Those women were older than the others ($P = 0.0016$). One patient became pregnant while on treatment. Two women delivered healthy children after cyc withdrawal.

Conclusion: Ovarian failure developed in 15.8% of our patients. As describe in the literature, the age at cyc initiation appears to be a determinant risk factor. Pregnancy may occur during cyc therapy,

and thus, an effective contraception is mandatory. After cyc withdrawal, pregnancy is possible with a favourable outcome.

Keywords: Systemic Lupus Erythematosus; Ovarian Failure; Cyclophosphamide; Pregnancy.

Introdução

A ciclofosfamida, um derivado nitrogenado da mostarda sintetizado em 1958, é frequentemente empregue no tratamento de envolvimento orgânico grave do LES, particularmente na nefrite lúpica.[1,2] Regimes com ciclofosfamida mostraram-se mais eficazes do que glucocorticóides isolados, na preservação da função renal em doentes com nefrite lúpica severa.[3,4] A administração por pulsos endovenosos intermitentes tornou-se o tratamento *standard,* uma vez que se demonstrou ter eficácia semelhante e menor toxicidade do que a administração contínua por via oral. [5]

A ciclofosfamida tem também demonstrado eficácia no tratamento de outras manifestações do LES, como citopenias, lesão do sistema nervoso central, hemorragia pulmonar e vasculite.[6-8]

A toxicidade é considerável. Náuseas e vómitos, queda de cabelo e leucopenia são efeitos secundários relativamente frequentes mas transitórios ou preveníveis. Já o risco aumentado de infecções, particularmente ao *Herpes zoster*,[9] e a cistite hemorrágica merecem outra atenção. A cistite, mais frequente com a administração oral, pode evoluir para lesão maligna vários anos após descontinuação do tratamento, o que justifica vigilância regular.[10]

A teratogenicidade constitui um risco importante da ciclofosfamida, pelo que é imperioso garantir contracepção eficaz durante a sua utilização.[11] A toxicidade gonádica pode resultar em falência ovárica e infertilidade persistente, já que as células germinais do ovário não se reproduzem nem se regeneram. Dada a maior prevalência das indicações terapêuticas em mulheres jovens, este efeito acessório constitui um problema clínico de alta relevância para a doente e o médico. A libido e função sexual geralmente não são afectadas.[11]

O efeito citotóxico da droga a nível ovárico é potenciado pela diminuição que induz nos níveis de estradiol - daqui resulta um aumento dos níveis de FSH e LH que aceleram a formação de folículos «jovens», mais sensíveis à acção tóxica da ciclofosfamida. O défice de estrogénios justifica o aparecimento de sinais e sintomas de menopausa como amenorreia, hipoplasia endometrial, atrofia do epitélio vaginal e sintomas vasomotores. Histologicamente observa-se destruição dos folículos ováricos e fibrose das áreas intersticiais.[11]

A recuperação da função gonadal após tratamento com ciclofosfamida é imprevisível.[2] Contudo, a idade é importante: de acordo com a literatura, em mulheres com nefrite lúpica tratadas com ciclofosfamida, a IO ocorre em quase 100% daquelas com idade superior a 30 anos, em cerca de 50% entre os 20 e 30 anos, e em apenas 13% das doentes com idades inferiores a 20 anos. Doses cumulativas mais elevadas provocam IO com maior frequência.[12-20]

Não é claro, à luz da literatura, se a duração de amenorreia após ciclofosfamida é preditiva da recuperação da menstruação ou fertilidade, ou ainda se as doentes que voltam a menstruar têm risco aumentado de menopausa precoce.[21]

Neste contexto, analisámos retrospectivamente a nossa série de doentes pré-menopáusicas com lúpus eritematoso sistémico submetidas a tratamento endovenoso com ciclofosfamida no sentido de avaliar a ocorrência de insuficiência ovárica, identificar factores de risco para esta complicação e apreciar a ocorrência e viabilidade de gravidez durante e após o tratamento.

Material e Métodos

O estudo integrou todas as mulheres pré-menopáusicas com o diagnóstico de LES, segundo os critérios do *American College of Rheumatology* (ACR), submetidas a terapêutica endovenosa com ciclofosfamida, no Serviço de Reumatologia dos Hospitais da Universidade de Coimbra. Excluímos as portadoras de amenorreia secundária a outras causas. Os processos foram revistos e actualizados por entrevista, recolhendo informações relativas à data do início do tratamento; dados demográficos; hábitos tabágicos e alcoólicos; índice de massa corporal; história ginecológica e obstétrica; caracterização da doença (duração, critérios ACR, SLEDAI prévio ao tratamento); perfil de auto-anticorpos; idade no início da ciclofosfamida e motivo da sua utilização; número de pulsos, dose e tempo total do tratamento; terapêutica concomitante; efeitos secundários, particularmente a amenorreia.

Definimos insuficiência ovárica pela ausência de menstruação por um período igual ou superior

a 4 meses, confirmada por doseamentos hormonais (FSH, LH e Estradiol).

Foi usado o teste não paramétrico de qualidade de ajuste, Kolmogorov-Smirnov, comprovando a distribuição normal das variáveis, para uma probabilidade de 95%.

O teste T-Student para amostras independentes foi usado para comparar dados paramétricos entre o grupo que desenvolveu IO e o que não apresentou esta complicação.

Resultados

Foram identificadas 19 mulheres seguindo os critérios acima expostos. A média de idades ao início do tratamento era de 28,4 ± 7,6 anos e a duração média da doença de 55,4 ± 55,6 meses. O valor médio do SLEDAI prévio ao tratamento era de 14,7 ± 5,7.

A glomerulonefrite lúpica, de diversas classes, foi a indicação para a terapêutica em 89,5% das doentes. As restantes duas doentes foram submetidas a este tratamento por envolvimento do sistema nervoso central (vasculite e nevrite óptica, respectivamente).

Todas as doentes foram submetidas a tratamento endovenoso com ciclofosfamida seguindo protocolo do *National Institute of Health* (NIH): 6 pulsos mensais na dose de 0,5-1 g/m^2, seguidos de 6 trimestrais, interrompidos apenas no caso de surgirem complicações graves ou ausência de resposta clínica.[20] Pulsos endovenosos de metilprednisolona (1g/dia, 3 dias) foram administrados a 57,9 % das doentes, imediatamente antes do primeiro pulso de ciclofosfamida. Todas elas estavam medicadas com corticosteróides orais diários, em doses de 5 a 20 mg de prednisolona. Todas as doentes tinham sido instruídas a realizar contracepção eficaz e 15,8% fizeram-no com contraceptivos orais (CO). Duas doentes foram medicadas com análogos LHRH para protecção ovárica.

As doentes receberam em média 9,3±3,4 pulsos endovenosos de ciclofosfamida ao longo de 16,5 ± 8,3 meses, com uma dose cumulativa média de 6.973 ± 2.611 mg.

Três doentes (15,8%) desenvolveram insuficiência ovárica. Destas, nenhuma voltou a menstruar durante o período de seguimento. Três mulheres referiram irregularidades menstruais com períodos de amenorreia inferior a 4 meses e as restantes negaram alterações menstruais (Figura 1 e Quadro I).

As doentes que sofreram IO apresentavam uma média de idades mais elevada aquando do início do citotóxico comparativamente com o outro grupo (41,3 ± 3,5 anos *versus* 26 ± 5,5 anos; $P = 0,0016$). Assim, observámos IO em 60% das mulheres com mais de 35 anos e em nenhuma das mais jovens. A média de idades ao início do lúpus foi também mais elevada nas mulheres que sofreram IO (36,3 ± 5,8 anos *versus* 22,4 ± 5,8 anos; $P = 0,03$). A dose cumulativa de ciclofosfamida nestas doentes não foi significativamente superior à verificada naquelas que não sofreram IO (7.917 ± 2.788 mg *versus* 6.796 ± 2.634 mg; $P = 0,28$).

Não encontramos diferenças significativas entre o grupo de doentes que desenvolveu IO e as restantes, no que diz respeito à actividade e à duração do LES ($P = 0,3$ e 0,4, respectivamente). As diferenças entre os valores de índice de massa corporal, carga tabágica e consumo de álcool também não apresentaram significado estatístico ($P > 0,5$).

Das onze mulheres que tentaram engravidar antes de iniciar o tratamento com ciclofosfamida, todas, com excepção de uma, tiveram êxito em pelo menos uma gravidez. Quatro referiram abortos espontâneos no 1º trimestre, sendo três portadoras de anticorpos anti-fosfolípidos.

No Quadro II são apresentadas as características principais das três doentes que desenvolveram IO, verificando-se que a amenorreia surgiu nessas mulheres, após o 1º, o 4º e o 5º pulso, respectivamente.

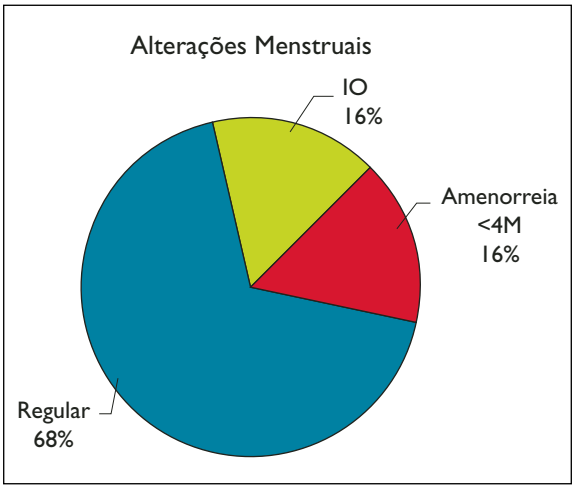

Figura 1. Percentagem de doentes com alterações menstruais.
IO: Insuficiência Ovárica

Quadro I. Caracterização das doentes, de acordo com o estado menstrual após tratamento com Ciclofosfamida.

	Total doentes N= 19	IO N= 3	S/ IO N= 16
IMC	23 ± 3,3	24,2 ± 5,2	22,8 ± 3,1
Tabaco (UMA)	0,7 ± 1,9	1,3 ± 2,3	0,6 ± 1,9
Álcool (g/dia)	3,2 ± 10	6,7 ± 11,5	2,5 ± 10
Idade início LES (A)	24,6 ± 7,8	36,3 ± 5,8	22,4 ± 5,8
Duração doença (M)	55,4 ± 55,6	60 ± 36	54,5 ± 59,5
Idade início Ciclof. (A)	28,4 ± 7,6	41,3 ± 3,5	26 ± 5,5
SLEDAI	14,7 ± 5,7	13,7 ± 3,2	15 ± 6,1
ANA (%)	19 (100)	3 (100)	16 (100)
Ac anti dsDNA	19 (100)	3 (100)	16 (100)
Ac anti RNP	6 (31,6)	1 (33,3)	5 (31,3)
Ac anti Ro/SSA	7 (36,9)	1 (33,3)	6 (37,5)
Ac anti La/SSB	1 (5,3)	1 (33,3)	0
Contraceptivo Oral (%)	3 (15,8)	0	3 (18,6)
Análogos LHRH (%)	2 (10,5)	0	2 (12,5)
Indicação Ciclof: (%)			
Glomerulonefrite	17 (89,5)	3 (100)	14 (87,5)
Envolvimento SNC	2 (10,5)	0	2 (12,5)
Nº Pulsos	9,3 ± 3,4	9 ± 3,6	9,3 ± 3,5
Tempo Total (M)	16,5 ± 8,3	15,7 ± 9,7	16,7 ± 8,4
Dose Cumulativa (mg)	6.973 ± 2.611	7.917 ± 2.788	6.796 ± 2.634

LES: Lúpus Eritematoso Sistémico; IO: Insuficiência Ovárica; Ciclof: Ciclofosfamida; Ac: Anticorpo; SNC: Sistema Nervoso Central; A: Anos; M: Meses; IMC: índice de massa corporal; UMA: unidades maço ano.

Durante o período de tratamento uma doente engravidou, tendo sido realizado aborto terapêutico às 6 semanas. Duas tiveram filhos saudáveis, 3 e 5 anos, respectivamente, após o término da terapêutica com ciclofosfamida.

Relativamente a outros efeitos secundários associados a este imunossupressor: 57,9% das doentes apresentaram queda de cabelo e 47% referiram intolerância gastrointestinal. Constatámos leucopenia em 10,5% das doentes. Ocorreram duas infecções oportunistas, uma por vírus *Herpes zoster* com envolvimento de dois dermátomos e outra por *Cândida albicans* com envolvimento oral e esofágico, ambas com necessidade de tratamento hospitalar. 26,3% das pacientes relataram outros efeitos adversos, nomeadamente, cefaleias, astenia, mal-estar geral, artralgias e febre (Figura 2).

Discussão

Estudos controlados demonstraram ser rara a ocorrência de IO, em mulheres pré-menopáusicas com LES não tratadas com ciclofosfamida, o que sugere que essa situação seja condicionada fortemente pelo efeito gonadotóxico deste agente alquilante e não pela actividade da doença ou utilização de outros fármacos, nomeadamente corticosteróides. [14-16]

A frequência da insuficiência ovárica induzida pela ciclofosfamida endovenosa encontrada nas nossas doentes (15,8%) é inferior à observada em outros estudos[3,12,14, 15,28,37] (Quadro III). Esta situação poderá justificar-se pelo tamanho da nossa amostra, uma vez que o esquema terapêutico usado foi sobreponível ao desses estudos, assim como a média de idade das doentes.

Outros investigadores usando regimes de administração semelhantes e com amostras mais numerosas demonstraram que a idade e a dose cumulativa do fármaco são factores de risco independentes para o desenvolvimento de insuficiência ovárica.[12-17] No nosso estudo, a idade mais elevada associou-se de forma estatisticamente significativa ao aparecimento de insuficiência ovárica.

Os nossos resultados não são concordantes com os de outros estudos [12-17,28] que mostram uma associação entre a dose cumulativa e a ocorrência de IO. Com efeito, para além da diferença entre a dose cumulativa nos dois grupos não ter apresentado significado estatístico, na nossa série os três casos de IO ocorreram precocemente (ao fim de 1, 4 e 5 meses de tratamento, respectivamente), ou seja, nessas doentes com idade mais elevada, doses cumulativas baixas (1.500, 3.800 e 5.000 mg, respectivamente) à data da instalação da amenorreia, foram suficientes para induzir IO (Quadro II).

Ioannidis e colaboradores[22] encontraram relação entre a presença de anticorpos anti-Ro e anti-U1RNP, bem como uma maior duração da doença, com o risco de insuficiência ovárica em doentes com idade inferior a 31 anos. De acordo com os seus resultados, a probabilidade de amenorreia persistente aumenta 1,33 vezes por cada ano adi-

cional de duração do lúpus, até ao início do tratamento, e de 11 vezes na presença de anticorpos anti--U1RNP. Concluíram também que 90% das doentes com mais de 32 anos desenvolvem insuficiência ovárica no caso de receberem dose de ciclofosfamida superior ou igual a 12g/m^2 e que, para doentes com essa idade ou mais velhas, a dose cumulativa deverá ser inferior a 5g/m^2, para obviar esta complicação.

Quadro II. Caracterização das doentes com Insuficiência Ovárica.

	Doente 1	Doente 2	Doente 3
Idade início Ciclof. (A)	38	41	45
Δt Ciclof-Amenorreia (M)	4	1,5	5
Tempo Tot Ciclof. (M)	5	24	18
Dose até amenorreia (mg)	3.800	1.500	5.000
Dose Total (mg)	4.750	9.000	10.000
FSH / LH / Estradiol (pg/ml)	96/83/<10	46/32/<10	62/51/<10
Amenorreia pós Ciclof (M)	6	48	48

Ciclof.: Ciclofosfamida; Δt: Intervalo de tempo; A: Anos; M: Meses

No nosso estudo não foi possível estabelecer uma correlação entre a IO e auto--anticorpos específicos, quer pelo pequeno número da amostra, quer pelo facto de alguns auto-anticorpos não terem sido pesquisados em todas as mulheres.

A fertilidade em mulheres com LES é considerada normal, sendo o principal problema reprodutivo, a ocorrência de perdas fetais ou abortos.[23] No entanto, poderá existir disfunção ovárica subclínica. Esta será mais proeminente em doentes com maior duração da doença e poderá estar associada à presença de anticorpos específicos.[24,25]

Na nossa amostra, dez das onze doentes que tentaram engravidar tinham filhos e quatro referiram abortos no 1º trimestre da gravidez. Duas mulheres tiveram filhos saudáveis após o tratamento e uma outra engravidou durante o período de terapêutica com a ciclofosfamida, confirmando a observação de que a gravidez é possível após ciclofosfamida e com prognóstico favorável.[16,26,27]

Contrariamente ao constatado em alguns estudos prévios,[12,14,22,28] no nosso estudo não encontramos associação entre a actividade ou a duração do LES e o desenvolvimento de insuficiência ovárica. O tempo médio desde o início da ciclofosfamida até ao desaparecimento da menstruação foi de cerca de 3,5 meses, concordante com a literatura. [14,15,28]

O citocromo P450 é essencial para activação da ciclofosfamida. Num estudo recente sobre farmocogenética desta enzima em doentes com nefrite lúpica tratados com a ciclofosfamida conclui-se que doentes homozigóticos para as variantes CYP2B6*5 e CYP2C19*2 terão maior probabilidade de má resposta renal ao tratamento e doentes homozigóticos ou heterozigóticos para a variante CYP2C19*2 terão um risco menor de insuficiência ovárica prematura.[29] Estas observações abrem a possibilidade que estudos de genotipagem possam ser úteis na identificação de doentes com maior risco para toxicidade ovárica, candidatas preferenciais às medidas protectoras.[16,26,30]

Os nossos resultados demonstram um risco elevado de insuficiência ovárica em mulheres pré--menopáusicas, com idade superior a 35 anos, sob terapêutica com ciclofosfamida.

Caso a manutenção da fertilidade seja imperiosa nestas mulheres, todas as hipóteses devem ser ponderadas, considerando a actividade do LES, as patologias associadas, a presença de anticorpos antifosfolípidos e a história obstétrica/ginecológica. As hipóteses passarão por estratégias de protecção ovárica, pela redução da dose e/ou tempo de

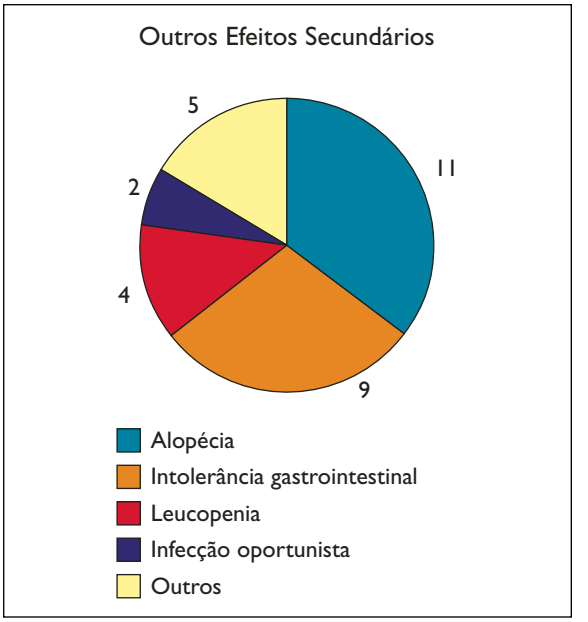

Figura 2. Nº de doentes com outros efeitos secundários ao tratamento com Ciclofosfamida (N=19).

Quadro III. Taxa de insuficiência ovárica em doentes com LES após tratamento com Ciclofosfamida.

Autores e referência	Nº doentes	Idade	Dose de ciclofosfamida	Insuficiência ovárica (%)
Boumpas[3]	13	28	14 pulsos de 0,5-1g/m^2	5 (38)
Boumpas[12]	13	29	7 pulsos de 0,5-1g/m^2	2 (15)
	14		15 pulsos de 0,5-1g/m^2	5 (35,7)
McDermont & Powell[15]	35	28	11 pulsos de 1g	19 (54)
Mok[14]	54	27	1-2 mg/Kg, oral, 9 meses	16 (30)
	16		12 pulsos de 0,5-1g/m^2	2 (16)
Huong[28]	56	28	12 pulsos de 0,9g	13 (23,2)
Illie[37]	23	28	14 pulsos 1g/m^2	12 (54)

tratamento com ciclofosfamida ou ainda pelo uso de terapêuticas alternativas (Imunoglobulina ev, micofenolato de mofetil, etc).[31-33] Wetzels, revendo a literatura sobre toxicidade gonadal induzida pela ciclofosfamida no tratamento da nefrite lúpica,[34] conclui que regimes prolongados (NIH) não são superiores no controlo da doença em relação a regimes mais curtos (Eurolupus), sendo estes menos gonadotóxicos.

Estudos experimentais e clínicos sugerem que análogos LHRH são eficazes na prevenção da IO.[16,21,26,27,30,35] Blumenfeld e colaboradores[27] verificaram, em estudo prospectivo e controlado, que a associação de análogos LHRH ao tratamento com ciclofosfamida em doentes com LES, preserva a função ovárica das mulheres em idade reprodutiva. Os resultados seguem o verificado em mulheres tratadas por linfoma e leucemia, nas quais os análogos LHRH reduzem a ocorrência IO de mais de 50%, para cerca de 4%.[16,30,36] Este efeito parece mediado por mecanismos complexos que incluem a supressão da libertação central de gonadotropinas e diminuição dos seus efeitos a nível gonádico. Daqui resulta um estado de quiescência folicular que confere maior resistência à toxicidade local da ciclofosfamida.[35] Outros autores sugerem que o efeito protector se deve, essencialmente, ao meio hipoestrogénico gerado por estas drogas que conduz a diminuição temporária da perfusão ovárica e consequente redução da exposição dos folículos ováricos ao agente alquilante.[27] Este meio *per se*, poderá também diminuir o risco de agudizações do LES.[37] Os análogos LHRH induzem amenorreia, que surge 3 a 8 semanas após o início da administração e cede 6 a 10 semanas após o seu fim.[21] 75% das doentes expostas a este fármaco apresentam sintomas vasomotores.[21] Outro efeito secundário importante desta medicação é a diminuição da densidade mineral óssea (DMO) por défice de estrogéneos. [21] Alguns estudos realizados sobre esta matéria concluíram que a perda de massa óssea (variável de 2 a 11,8%), é parcialmente recuperável, desde que a duração do tratamento não exceda os 6 meses.[16,21,38]

As duas doentes que receberam análogos LHRH no nosso estudo não desenvolveram insuficiência ovárica. Contudo, este desfecho favorável pode estar relacionado com a idade destas doentes (21 e 22 anos), precisamente a razão por que foram eleitas para terapêutica preventiva. Em raparigas com idade inferior a 16 anos os análogos LHRH não são aconselhados pelo facto de se desconhecer o seu efeito no crescimento ósseo, devendo nessa altura ponderar-se a utilização de CO conjugados de baixa dosagem.[16,21]

A utilização de contraceptivos orais com o objectivo de prevenir a IO em doentes com LES, não é consensual, pelo risco de agravamento da doença. Em 1995, Sanchez-Guerrero e colaboradores demonstraram a existência de risco ligeiramente aumentado para desenvolver LES quer em mulheres pós-menopáusicas que recebem terapêutica hormonal de substituição[39] quer em mulheres utilizadoras de CO.[40] Em 1997, Petri e Robinson[41] concluíram que os CO deverão ser evitados em mulheres com LES e doença renal activa, pelo risco aumentado de «flare». Em estudos mais recentes, ambos os autores concluem que os CO não aumentam o risco de «flare» em doentes com LES inactivo ou estabilizado.[42,43] Embora a utilização de CO nas doentes com LES seja uma hipótese real que passará sempre pela avaliação cuidadosa dos riscos (agudização da doença e ocorrência de acidentes trombóticos em doentes portadores de an-

ticorpos anti-fosfolípidos) contra os benefícios (contracepção, preservação da DMO e possivelmente da função ovárica), a sua utilização no LES com actividade e envolvimento de órgão-*major,* requerendo a utilização de ciclofosfamida, está limitada.

Outras vias de tratamento sugeridas para a preservação da fertilidade em mulheres expostas a gonadotóxicos durante a idade reprodutiva, incluem a criopreservação de óvulos, tecido ovárico ou mesmo embriões.[16] Estas propostas são controversas, quer pelas questões técnicas e éticas que suscitam, quer pelo facto da estimulação ovárica pela hMG/hCG, prévia à colheita de óvulos, causar elevação do estradiol que poderá agravar a situação clínica do LES.[16] Foram publicados recentemente os primeiros resultados, com sucesso, da utilização destas vias alternativas em doentes com patologias oncológicas.[44,45]

São necessários mais estudos sobre a IO induzida pela ciclofosfamida em doentes com LES e, principalmente, estudos sobre estratégias que a possam evitar.

O Grupo Alemão de Estudos para o Linfoma de Hodgkin tem em curso um ensaio randomizado em fase II utilizando CO e análogos LHRH como terapêutica profilática da insuficiência ovárica, em mulheres jovens submetidas a quimioterapia por essa doença. Aguardamos com entusiasmo estes resultados que poderão definir um tratamento adjuvante *standard* que diminua a taxa de infertilidade nessas e nas nossas doentes com LES.[46]

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RECOMMENDATIONS FOR THE DIAGNOSIS AND TREATMENT OF LATENT AND ACTIVE TUBERCULOSIS IN INFLAMMATORY JOINT DISEASES CANDIDATES FOR THERAPY WITH TUMOR NECROSIS FACTOR ALPHA INHIBITORS – MARCH 2008 UPDATE

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Abstract

The Portuguese Society of Rheumatology and the Portuguese Society of Pulmonology have updated the guidelines for the diagnosis and treatment of latent tuberculosis infection (LTBI) and active tuberculosis (ATB) in patients with inflammatory joint diseases (IJD) that are candidates to therapy with tumour necrosis factor alpha (TNFα) antagonists. In order to reduce the risk of tuberculosis (TB) reactivation and the incidence of new infections, TB screening is recommended to be done as soon as possible, ideally at the moment of IJD diagnosis, and patient assessment repeated before starting anti-TNFα therapy. Treatment for ATB and LTBI must be done under the care of a TB specialist. When TB treatment is indicated, it should be completed prior to starting anti-TNFα therapy. If the IJD activity justifies the need for immediate treatment, anti-TNFα therapy can be started two months after antituberculous therapy has been initiated, in the case of ATB, and one month after in the case of LTBI. Chest X-ray is mandatory for all patients. If Gohn's complex is present, the patient should be treated for LTBI; healed lesions require the exclusion of ATB. In cases of suspected active lesions, ATB should be excluded/confirmed and adequate therapy initiated.

Tuberculin skin test, with two units of RT23, should be performed in all patients. If the induration is <5 mm, the test should be repeated within 1 to 2 weeks, on the opposite forearm, and will be considered negative only if the result is again <5 mm. Positive TST implicates LTBI treatment, unless previous proper treatment was provided. If TST is performed in immunossuppressed IJD patients, LTBI treatment should be offered to the patient before starting anti-TNF-α therapy, even in the presence of a negative test, after risk/benefit assessment.

Keywords: Guidelines; Portuguese Society of Rheumatology; Portuguese Society of Pulmonology; Tuberculosis; Anti-TNFα drugs

Resumo

A Sociedade Portuguesa de Reumatologia e a Sociedade Portuguesa de Pneumologia actualizaram as recomendações para o diagnóstico e terapêutica da tuberculose latente (TL) e activa (TD) em doentes com doenças inflamatórias articulares (DIA), candidatos a tratamento com antagonistas do factor de necrose tumoral alfa (TNFα).

Com o objectivo de reduzir o risco de reactivação da tuberculose (TB) ou nova infecção, recomenda-se o rastreio de TD e TL tão precocemente quanto possível, preferencialmente no momento do diagnóstico da DIA, e repetir a avaliação do doente antes de iníciar terapêutica anti-TNFα. O tratamento da TD e TL deve ser sempre supervisionado por um especialista em TB. Quando houver indicação para terapêutica de TB, esta deverá ser cumprida integralmente antes de se iniciar o anti-TNFα. No caso da actividade da DIA o exigir, o anti-TNFα poderá ser iniciado após dois meses de terapêutica antibacilar, no caso de TD, ou após um mês, no caso de TL.

Todos os doentes devem realizar radiografia do tórax. Alterações compatíveis com complexo de

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Gohn devem ser tratadas como TL. Lesões residuais obrigam a excluir TB activa. Se se suspeitar de lesões em actividade, o diagnóstico de TD deve ser excluido e o tratamento adequado instituído.

A prova tuberculínica (PT), com 2 Unidades de Tuberculina RT23 deverá ser efectuada em todos os doentes. Se a indução for <5 mm, a prova deve ser repetida dentro de 1 a 2 semanas, no antebraço oposto, e considerada negativa apenas se o segundo resultado for igualmente <5 mm. As PT positivas obrigam a tratamento de TL, excepto se o doente tiver sido previamente tratado de forma adequada. Se a PT é realizada apenas em fase de imunodepressão, mesmo que seja negativa, deve ser equacionado o tratamento de TL antes de iniciar terapêutica anti-TNFα, após ponderar a relação risco/benefício.

Palavras-chave: Guidelines; Sociedade Portuguesa de Reumatologia; Sociedade Portuguesa de Pneumologia; Tuberculose; Anti-TNFα

Introduction

Tumor necrosis factor alpha (TNF-α) inhibitors are used to treat inflammatory joint diseases (IJD) such as rheumatoid arthritis (RA), psoriatic arthritis (PsA) and ankylosing spondylitis (AS). In populations with a high incidence of tuberculosis (TB), there have been an increased number of TB cases reported in patients undergoing these therapies.[1] In fact, the relative risk (RR) of developing TB is 19 times higher in RA patients under anti-TNF-α therapy than in RA patients not undergoing such therapy.[1] However, it is important to point out that RA patients treated with conventional immunosuppressive drugs have a RR for TB that is 4 times higher than in the general population.[1]

In patients treated with anti-TNF-α drugs, ATB usually results from the reactivation of a latent infection. TB onset usually occurs during the first months of treatment and often presents an atypical behavior, which may pose difficulties to the diagnosis.[2] In countries with high incidence of TB, cases caused by new infections are particularly frequent. TNF-α is fundamental for the immunological defence against *Mycobacterium tuberculosis*, specially in the formation and maintenance of granulomas. Animal models show that it is possible to reactivate TB after administering anti-TNF-α antibodies.[3]

The currently available anti-TNF-α drugs are adalimumab, etanercept and infliximab. These three drugs have been approved for use in RA, AS, PsA and psoriasis. In addition, etanercept has also been approved for use in juvenile idiopathic arthritis and infliximab and adalimumab for use in Crohn's disease.

Because of its greater epidemiological impact, as compared to other IJDs, RA has been considered a model for introducing new biotechnology derived drugs that interfere with the immune system.[4,5] RA affects around 1% of the world population and might have a very aggressive course, leading to disability, increased co-morbidity and mortality.[6,7] For this reason, the use of disease modifying anti-rheumatic drugs – DMARDs - should be started as early as possible and methotrexate (MTX) is the first treatment option for this therapeutic strategy. However, in cases where MTX is contra-indicated or where it is impossible to use an adequate dose due to intolerance or toxicity, other immune modulating drugs may be used, such as leflunomide, cyclosporine and sulphasalazine. In patients who continue to present active disease, despite of the use of MTX at the maximum tolerated dose, alternative therapeutic measures should be taken, specifically the introduction of an anti-TNF-α drug. This approach is supported by international recommendations[8] and by the guidelines for using biologic agents in RA therapy[9] published by the Rheumatoid Arthritis Study Group (Grupo de Estudos de Artrite Reumatóide- GEAR) of the Portuguese Society of Rheumatology (SPR). The Portuguese experience on the use of biologic therapies in the treatment of RA has been recently reviewed by the SPR.[10] The SPR also sponsored guidelines for initiating biologic therapy in AS[11] and published the Portuguese experience on the use of biologic therapies in the treatment of this disease.[12] There are no specific guidelines on when to start biologic therapy in PsA, but in general, polyarticular forms similar to RA are treated according to the SPR recommendations for RA, and cases that are predominantly axial are treated according to the SPR recommendations for AS.

Keeping patients on anti-TNF-α therapy depends on documenting its efficacy. The three TNF-α antagonists seem to display similar clinical effectiveness in the diseases mentioned above. However, there are differences from the molecular point of view, and the mechanisms whereby each drug acts are not entirely equal. Etanercept is a di-

meric fusion protein consisting of the extracellular ligand of the p75 TNF receptor combined with the Fc portion of human IgG1. It forms stable bonds with the soluble trimeric forms of TNF-α and TNF-β (lymphotoxin), keeping them from interacting with their respective receptors. It also interacts with monomeric TNF-α and with transmembrane TNF-α, but in this case the affinity is low and has a transient effect (90% of trans-membrane TNF-α is released from the etanercept binding within 10 minutes). Etanercept is administered at a dose of 25 mg twice weekly or 50 mg once weekly, as a subcutaneous (SC) injection. Etanercept may be used for RA treatment as monotherapy or in association with MTX (in this case the literature reports enhanced effectiveness).[13,14] It has been approved as monotherapy against AS and PsA,[14] but is generally used together with MTX to treat PsA. Infliximab is a chimeric monoclonal antibody with a high affinity and specificity for TNF-α, forming stable complexes with monomeric and trimeric TNF-α, and with trans-membrane TNF-α. Infliximab has no affinity for TNF-β (lymphotoxin). The binding to trans-membrane TNF-α induces cell lyses, mediated by complement dependant cytotoxicity or antibody dependent cellular cytotoxicity. It can also induce apoptosis by mechanisms that are not fully understood, but known to involve the caspases.[15] These cellular effects lead to a reduction in the number of TNF-α producing cells (monocytes and CD4 and CD8 lymphocytes), that is not observed with etanercept.[16] Infliximab is administered intravenously in doses that vary according to the disease and clinical response.[17] In RA it is used in association with MTX, usually in doses of 3 mg/kg every 8 weeks.[17] In PsA it is used in association with MTX, usually in doses of 5 mg/kg every 8 weeks.[17] In AS, it is used as monotherapy in doses of 5 mg/kg every 6 weeks.[17] Adalimumab is a human recombinant monoclonal IgG1 antibody, with a mechanism of action similar to infliximab. It is administered subcutaneously at 40 mg doses every other week.[18] Adalimumab may be used for the treatment of RA as monotherapy or in association with MTX[18] (in this case the literature reports enhanced effectiveness).[19] Adalimumab has been approved for use as monotherapy[18] in PsA and AS.

In the US, where the annual incidence of TB is 6.2 cases per 100,000 inhabitants, the incidence of TB in patients treated with infliximab is 54 per 100,000 and, with etanercept, 28 per 100,000. This difference may merely be due to the different risk of reactivating TB in the populations exposed to these two anti-TNF-α drugs.[20] However, the different mechanism of action could also explain the lower risk of TB reactivation in patients treated with etanercept. In fact, the above described effect of anti-TNF-α monoclonal antibodies on the cells expressing TNF-α, and their ability to irreversibly inhibit receptor p75 and p55 signaling, constitutes an hypothetical reason for a reduced preservation of granuloma integrity during continuous therapy with infliximab and adalimumab.[21]

Since 2002, specific guidelines for screening candidates to anti-TNF-α therapy for active and latent TB[9] have been followed. However, in spite of this, there have been cases of TB in this group of patients, especially among those on monoclonal antibody therapy (infliximab and adalimumab). This has been observed in Portugal in a study performed by the SPR.[22]

The incidence of TB in the general Portuguese population (29.4/100,000 inhabitants in 2006)[23] is much higher than the rate in the US and in most European countries. This fact requires that the international recommendations concerning screening and treating these patients for TB should be adapted to the Portuguese reality.

For these reasons, SPR's GEAR and the Tuberculosis Committee (TC) of the Portuguese Pulmonology Society (SPP) have elaborated recommendations for the diagnosis and treatment of LTBI and ATB in IJD patients treated with anti-TNF-α and other immunosuppressant drugs.

The main objective of these recommendations is to contribute for the reduction of the number of cases of reactivated TB and new TB infections in patients who are candidates for treatment with TNF-α antagonists in Portugal. An additional objective is also to standardize the procedures used to screen and prevent tuberculosis in the initial assessment of IJD patients, preferably before the onset of any immunosuppressant therapy.

A group of experts was appointed by SPR's GEAR and SPP's TC to develop these recommendations. The group made an extensive review of the literature using the *PubMed/Medline* search engine and the following keywords: tuberculosis and TNF-α antagonists – 199 publications; tuberculosis and infliximab – 184 publications, tuberculosis and adalimumab – 48 publications, tuberculosis and etanercept – 89 publications, isoniazid and methotrexate – 26 publications. In addition, the group reviewed the following information: TB in patients

undergoing anti-TNF therapy,[22] data on TB[23] published by the General Directorate of Health («Direcção Geral de Saúde»), national standards for the treatment of LTBI[24] and the international recommendations for screening and preventing TB in candidates for TNF-α antagonists therapy.[25-28] The recommendations were also based on the operational capacity of the Pulmonology Diagnostic Centers (Centros de Diagnóstico Pneumológico-CDP) and the Pulmonology and Rheumatology Departments. When the recommendations were ready, but before the final document was concluded, they were submitted to the review of two Spanish specialists responsible for the evaluation of the effectiveness of the Spanish recommendations to prevent reactivation of latent tuberculosis infection in patients treated with tumor necrosis factor antagonists (LC and JGR)[26], and also to a specialist in infectious diseases experienced in the treatment of tuberculosis (Dr. Germano do Carmo). The recommendations were presented and submitted to a public debate at a round table organized specifically for this purpose in the Portuguese Rheumatology Congress (April, 2006) and during a national meeting in the World TB Day (March, 2006).

In June 2006, the initial draft version of the recommendations was placed on the SPR Internet website to enable further public debate. The recommendations included the suggestions and criticisms received as part of this final public debate process.

The first version of these guidelines was published in September 2006[29,30] and placed on the General Directorate of Health Internet website in December 2006. This first review was based on the clinical experience obtained on the use of these recommendations during 2007 and on new data recently published.

The applicability, effectiveness and safety of these recommendations will be regularly reviewed by the Sponsoring Societies (SPR and SPP); the recommendations will be reviewed whenever warranted by new data or evidence.

Recommendations

Due to the compromised immunity observed in RA and other IJD patients, as a consequence of the physiopathology of the disease itself and of immunosuppressant therapies,[26] rheumatologists should screen for ATB and LTBI (Fig. 1) as early as possible, preferably at the moment of the diagnosis of an IJD. The objective is to obtain an evaluation of each patient before any immunosuppressant therapy is started. At this time the diagnostic accuracy of the tuberculin skin test is similar to the one obtained in the general population, thus enabling the treatment to be focused on the individuals with the highest probability of having LTBI. However, even if patients have been screened at the onset of disease, screening should be repeated before starting treatment with anti-TNF-α. If the initial assessment was negative, the second screening will recognize any LTBI or ATB that may have occurred in the meantime. In individuals that have been treated for LTBI before, this second screening will identify any possible ATB. If correctly performed, LTBI treatment should only be done once in the lifetime.

These patients should be assessed based on their clinical history, focusing on TB risk factors, as well as complementary tests to detect ATB or LTBI.

The following situations should be referred to a CDP:

- Patients with indication for treatment of LTBI or ATB,
- Patients with symptoms suggesting ATB,
- Patients at high risk for TB,
- Patients with Gohn's complex or healed lesions on chest X-rays and a history of untreated TB,
- Patients with a positive tuberculin skin test,
- All patients with IJD patients who are anti-TNF-α candidates and are already immunossuppressed.

The tuberculin skin test should be performed and interpreted in a CDP, whenever possible.

When treatment for tuberculosis (LTBI or ATB) is indicated, this should preferably be completed before starting anti-TNF-α. However, If the IJD activity justifies the need for immediate treatment, anti-TNF-α therapy can be started two months after the beginning of antituberculous therapy in the case of ATB, and one month after the beginning of antituberculous therapy in the case of LTBI.[25, 31, 32]

Clinical history

a) Symptoms suggestive of ATB → if yes, refer to CDP
b) Check personal history for TB risk factors: → if yes, refer to CDP
   i. previous TB
   ii. recent immigrants coming from countries with a high incidence of TB
   iii. recent contact with infectious patients
   iv. health care professionals
   v. IV drug users

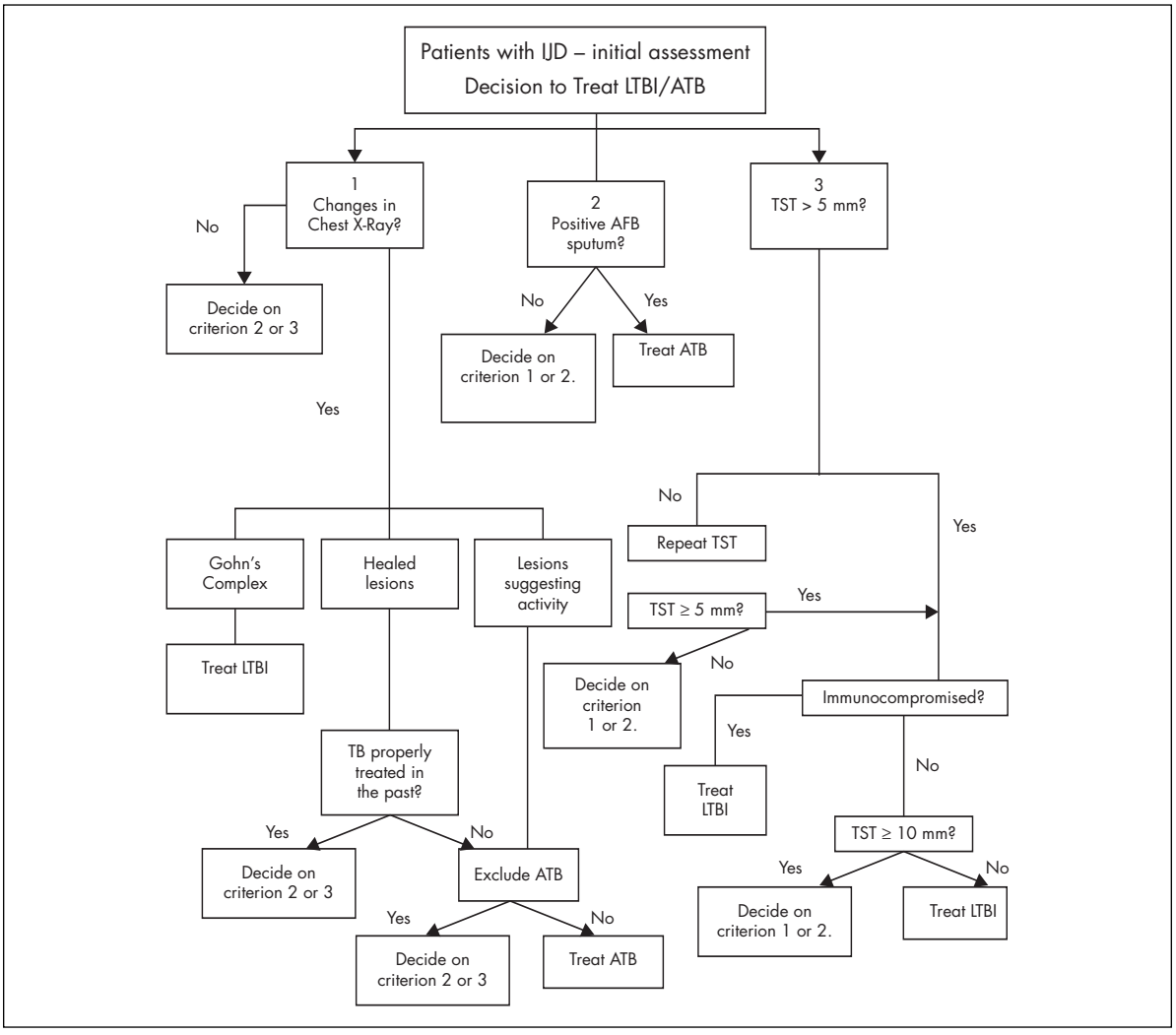

Figura 1. Patients with IJD - initial assessment. Decision to Treat LTBI/ATB. If criteria 1, 2 and 3 are negative, do not treat for LTBI/ATB

AFB: Acid-fast bacilli; ATB: Active Tuberculosis; IJD: Inflammatory Joint Disease; LTBI: Latent Tuberculosis infection; TB: Tuberculosis; TST: Tuberculin skin Test.

vi. diabetes, HIV infection, leukemias, lymphomas, head, neck or lung cancer

Complementary Tests to be done

c) Chest X-Ray that can be:
   i. Normal
   ii. Abnormal:
      1. Gohn's complex → treat for LTBI, refer to CDP
      2. Fibrotic lesions
         a. Past history of properly treated TB → the decision will depend on other procedures
         b. Past history of untreated or incorrectly or incompletely treated TB → exclude ATB → treat for LTBI, refer to CDP
      3. Active lesions → confirm ATB diagnosis → treat for ATB, refer to CDP

   d) In the presence of symptoms or X--Ray findings suggestive of active tuberculosis, check for the presence of *Mycobacterium tuberculosis* (microscopic examination and culture of sputum and, if positive, perform drug susceptibility tests).
   e) Tuberculin skin test (TST) should be

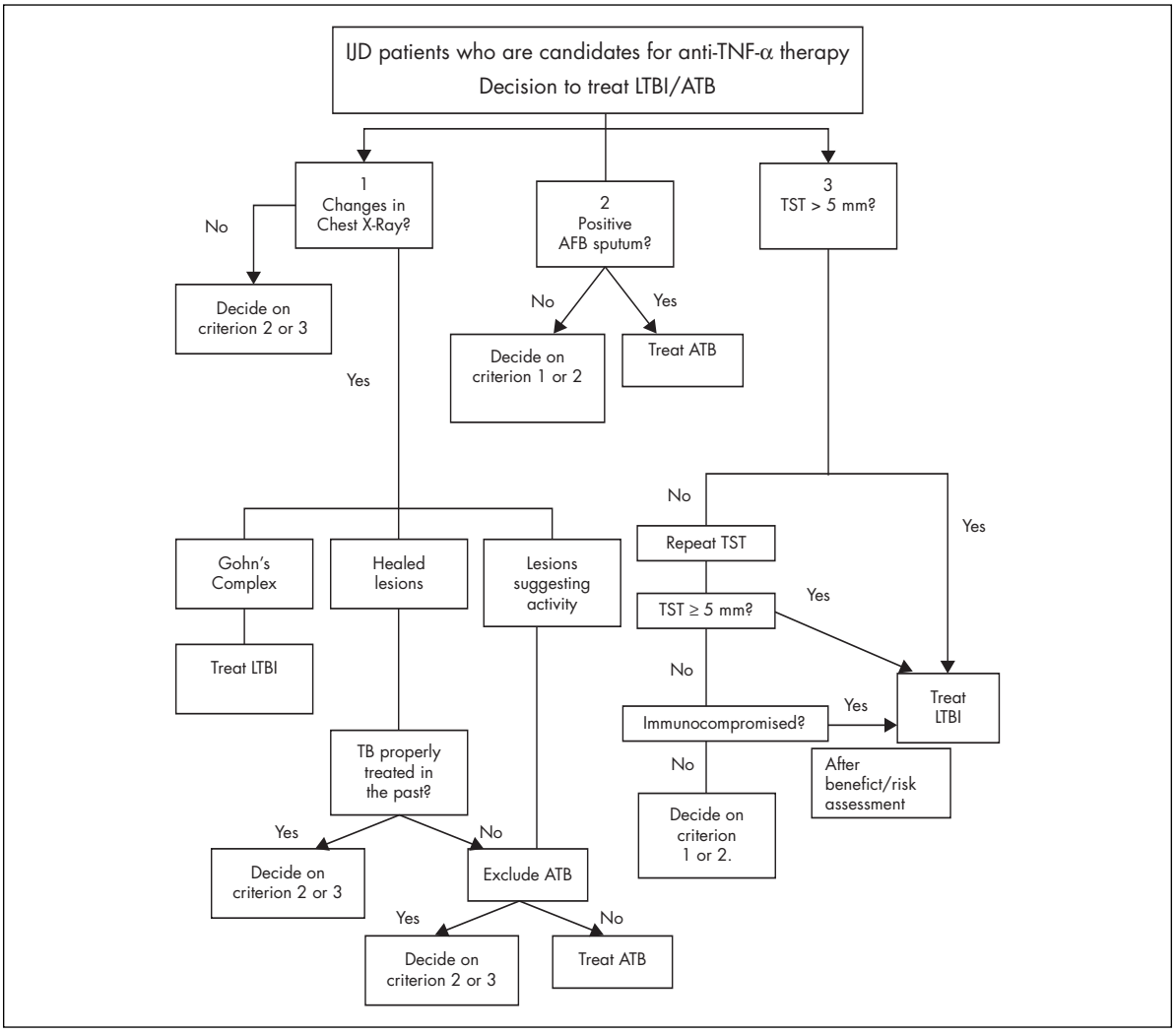

Figura 2. IJD patients who are candidates for TNF-α treatment Decision to treat LTBI/ATB. If criteria 1, 2 and 3 are negative, do not treat for LTBI/ATB.

AFB: Acid-fast bacilli; ATB: Active Tuberculosis; IJD: Inflammatory Joint Disease; LTBI: Latent Tuberculosis; TB: Tuberculosis; TST: Tuberculin Skin Test;

interpreted as follows:

i. < 5 mm – negative → repeat TST on the other forearm within the next 7 to 14 days. If the second test is positive (≥5 mm), only this last result should be used.
ii. ≥ 5 mm – positive in any patient who is about to start anti-TNF-α treatment or in the initial assessment of any IJD patient who fulfill the criteria for an immunossuppressed patient.
iii. ≥ 10 mm – positive in the initial assessment of any IJD disease and in patients who do not meet the criteria for an immunossuppressed patient.

Procedure following Tuberculin Test:

– if positive (ii and iii) → treat for LTBI (except if a correct treatment was performed in the past), refer to CDP
– if negative:
  a) Patient not previously exposed to immunosuppressive drugs → initiate anti-TNF-α
  b) Immunossuppressed patient → treat as LTBI, refer to CDP. This decision should be taken on an individual basis, after a benefit/risk as-

sessment, taking into account the age of the patient, ethanol consumption, previous hepatic diseases and the evaluation of LTBI risk.

Notes

- Immunossuppressed patients are those with established IJD, treated with steroids (prednisolone in doses higher than 10 mg/day) and/or with immunosuppressant drugs such as MTX, cyclosporine, azathioprine, leflunomide or cyclophosphamide, regardless of the dose.
- For immunossuppressed patients, a negative tuberculin skin test does not exclude TB. For this reason, if the tuberculin test is conducted in an immunossuppressed phase, the patient should be treated for LTBI before starting anti-TNF-α therapy, even if the test is negative. This decision should be taken on an individual basis, after a benefit/risk assessment, taking into account the age of the patient, ethanol consumption, previous hepatic diseases and the evaluation of LTBI risk.
- Some authors[33] suggest that tuberculin skin test should not be conducted on immunossuppressed patients who are candidates for treatment with TNF-α antagonists, given the decision to treat them for LTBI regardless of the result of the tuberculin skin test. These recommendations defend the tuberculin skin test, as this information may be useful in the future to determine the sensitivity and specificity of the tuberculin skin test in such patients, and may help to assess the impact of LTBI treatment based on the tuberculin skin test result.
- The threshold for considering a positive tuberculin skin test was reduced from 10mm to 5mm in patients who will start therapy with TNF-α antagonists, even in the absence of depressed immunity criteria, because of the high risk of developing serious forms of TB associated with the use of these drugs.

Treatment regimens for Latent Tuberculosis

a) Isoniazid for 6 months (6H) – 60% efficacy. Level of evidence: A[31]
b) Isoniazid for 9 months (9H) – 70% efficacy. Level of evidence: A[26]
c) Isoniazid and Rifampicin for 3 months (3HR) – 50% efficacy. Level of evidence: A[31]
d) Isoniazid, Rifampicin and Pyrazinamide for 2 months (2HRZ) – efficacy study currently underway. Level of evidence: D[34]

Treatment regimens for Active Tuberculosis

e) Isoniazid, Rifampicin, Pyrazinamide and Ethambutol for 2 months, followed by Isoniazid and Rifampicin for 4 months.[35,36]
f) Other regimens may be proposed in specific cases (co-morbidities, such as liver or kidney failure, or if drug susceptibility testing indicates resistance to some of the first-line drugs).

Comments

1. Patients should be screened and treated for LTBI or ATB when an IJD is first diagnosed and when the patient is a candidate for starting anti-TNF-α therapy.
2. Although prior screening is mandatory for all patients treated with TNF-α antagonists, none of the LTBI treatment regimens is 100% effective. In addition, patients contact with hospital environments, where there is a higher risk of contacting with TB patients, increasing the risk of new infections. For these reasons, patients should be carefully monitored for TB symptoms throughout the period they receive anti-TNF-α drugs and for six months after the drugs are discontinued. This clinical vigilance should be complemented, whenever necessary, by X-Rays and other suitable complementary diagnostic tests.[25]
3. Therapy for ATB should be administered under direct observation (DOT).
4. For TST it should be used 2 units of RT23 Tuberculin.
5. There are new tests to diagnose LTBI, such as γ-interferon quantification; their usefulness in immunossuppressed IJD patients is currently being assessed.
6. Treatment for ATB and LTBI must be done under the care of a TB specialist, who will also address all diagnostic or therapeutic questions.
7. There is toxicity, especially liver toxicity, associated with LTBI therapy. The risk of liver toxicity increases with age. There is little data available on the risk of liver toxicity in IJD patients treated

with DMARDs combined with anti-tuberculous drugs.[37,38] Patients should be carefully watched by a CDP specialist, both clinically and with laboratory tests, using the prevailing guidelines.[24]

8. In non-immunossuppressed patients, the risk of LTBI evolving to ATB is 10% over a patient's lifetime.[24,39,40] Treatment for LTBI reduces this risk to about 0.5%.[39,40]
9. In immunossuppressed patients, the risk of LTBI evolving to ATB is 8 to 10% per year.[41] In these patients, ATB may present atypically (making the diagnosis more difficult and often delayed) and it is generally more serious and associated with higher mortality rates.
10. LTBI therapy effect lasts for over 20 years. In fact, some authors even admit that the effect lasts for the patient's lifetime[41]. Because of this, patients are treated for LTBI only once. LTBI treatment plans and duration are identical for all patients, regardless of being immunossuppressed or not.

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Este artigo foi co-publicado no nº2 de 2008 da Revista Portuguesa de Pneumologia (Rev Port Pneumol 2008; XIV(2): 271-283); This article has been copublished in the issue no. 2 of the Revista Portuguesa de Pneumologia (Rev Port Pneumol 2008; XIV(2): 271-283)).

TUBERCULOSE PULMONAR E OSTEOARTICULAR

Anabela Barcelos,* Aida Coelho,** Filomena Freitas,*** Célia Oliveira***

Resumo

A prevalência da tuberculose tem vindo a aumentar nos últimos anos, em parte devido à pandemia da Sida. Apesar de ser vulgarmente considerada uma doença pulmonar, a tuberculose pode também atingir outros órgãos (sistema nervoso, génito-urinário, gastrointestinal, osteoarticular, etc) por disseminação linfo-hematogénia.

Os autores apresentam um caso de tuberculose pulmonar e osteoarticular diagnosticada simultâneamente.

Palavras-chave: Tuberculose; Tuberculose Pulmonar; Sacroileíte; Infecção Óssea.

Abstract

Tuberculosis is an important illness with an increasing prevalence in the last years in part due to AIDS. Considered primarily a pulmonary disease, tuberculosis can also affect almost any body system (nervous, genitourinary, and gastrointestinal as well as bones and joints) through lymphohematogenous spread.

The authors present a case of pulmonary and osteoarticular tuberculosis diagnosed simultaneously.

Keywords: Tuberculosis; Pulmonary Tuberculosis; Sacroiliitis; Infectious Bone Disease.

Introdução

A tuberculose é uma das doenças infecciosas cujo agente foi descoberto há mais tempo e que continua a atingir a Humanidade nos dias actuais. A Organização Mundial de Saúde (OMS) estima mesmo que a infecção tuberculosa exista em um terço da população mundial.[1]

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A taxa de incidência na população portuguesa de 29,4 casos por 100.000 habitantes (3.092 novos casos) em 2006[2] é muito superior à observada nos Estados Unidos da América (4,6 casos por 100.000 habitantes em 2006).

Considerada primariamente uma doença pulmonar, a tuberculose pode também atingir outros órgãos (sistema nervoso central, génito-urinário, gastrointestinal, osteoarticular, etc) por disseminação linfo-hematogénia, durante a infecção pulmonar inicial.[3] O atingimento ósseo e articular pelo *Mycobacterium tuberculosis* representa 10% dos casos de tuberculose extra-pulmonar ou aproximadamente 2% de todos os novos casos.[4-6]

Em áreas não endémicas, a tuberculose osteoarticular tem maior prevalência nos idosos enquanto que as crianças e os adultos são mais afectados em áreas endémicas.[7] A tuberculose osteoarticular envolve a coluna vertebral em 50% dos casos; com menor frequência a anca ou o joelho (30%) enquanto as articulações do punho, ombro e sacroiliaca são mais raramente afectadas (20% dos casos).[8] As manifestações clínicas são, na maioria das vezes, inespecíficas e insidiosas, dificultando o diagnóstico e instituição do tratamento adequado.[4-8] Em 1996, Teresa Martins *et al* publicaram uma casuística de 5 casos de tuberculose óssea em Portugal.[9]

A situação nacional da tuberculose é preocupante, apesar de nos últimos anos se terem registado progressos. Por um lado, o número total de casos novos de tuberculose tem crescido todos os anos e, por outro lado, as formas de tuberculose multirresistente e ultrarresistentes estão em progressão.[10]

Os autores apresentam um caso de tuberculose pulmonar e osteoarticular diagnosticado simultâneamente e chamam a atenção para a necessidade do diagnóstico e tratamento precoces da tuberculose pulmonar por forma a evitar a disseminação do *M. tuberculosis*.

Caso clínico

Doente de 34 anos, sexo feminino, raça negra, tra-

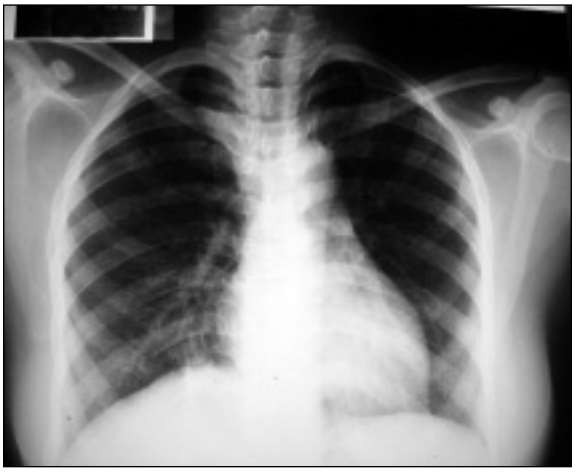
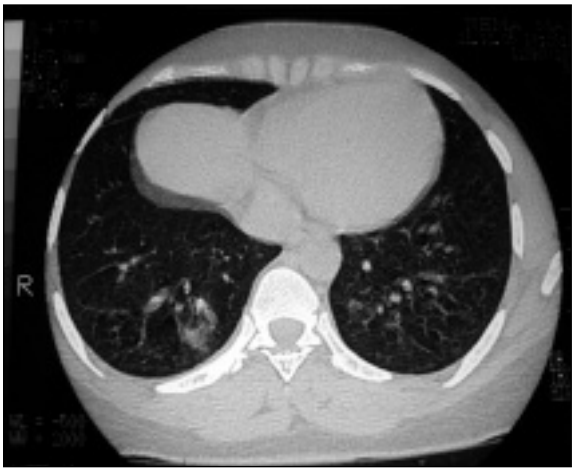


Figura 1. Rx do tórax – lesão infiltrativa discreta extendendo-se do hilo direito até à base do pulmão.



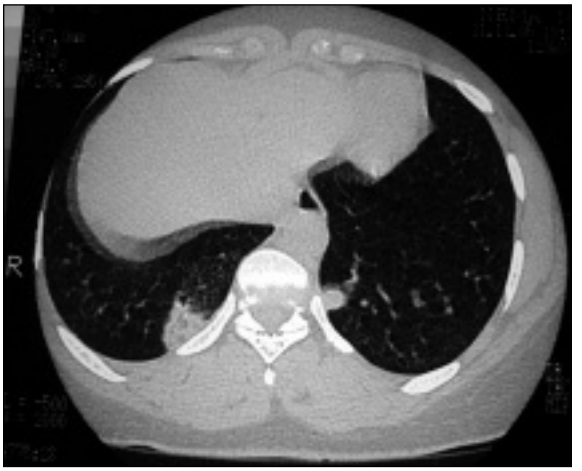


Figura 2 e 3. TAC torácica - focos de condensação no segmento basal posterior do lobo inferior direito e do lobo inferior esquerdo e numerosos pequenos micro-nódulos a nível dos andares superiores dos dois campos pulmonares.

balhadora fabril, natural da Guiné-Bissau, casada, sem antecedentes pessoais relevantes, internada para esclarecimento diagnóstico de Síndroma Febril Indeterminada.

Seis meses antes do internamento, a doente iniciou febre elevada e toracalgia que cedeu à instituição de antibioterapia (amoxicilina e ácido clavulânico 1g de 12/12 horas). Um mês depois recorre ao serviço de urgência do hospital da área de residência por febre elevada (38-40ºC) vespertina, acompanhada de sudorese nocturna e tosse com expectoração purulenta, sendo internada no serviço de Pneumologia para esclarecimento diagnóstico. Concomitantemente referia dor na região glútea esquerda e coxalgia à esquerda, de ritmo misto, por vezes com irradiação ao joelho homolateral e com exacerbação em decúbito lateral esquerdo. Foi observada por médico ortopedista e medicada com anti-inflamatório não esteróide (AINE) com melhoria. Foi-lhe diagnosticada patologia intersticial pulmonar sendo orientada para a consulta externa de Pneumologia. Uma semana depois reiniciou febre, sudorese nocturna, tosse produtiva, dor na região glútea e coxalgia à esquerda, condicionando vários despertares nocturnos com dores, e rigidez matinal axial superior a uma hora de duração. Negava emagrecimento ou anorexia. Foi novamente internada no Serviço de Pneumologia sendo solicitada a colaboração da Reumatologia e Infecciologia. Foi diagnosticada tuberculose pulmonar (baciloscopias positivas para *Mycobacterium tuberculosis*) e instituída terapêutica antituberculosa (isoniazida 150mg/dia, rifampicina 600mg/dia, pirazinamida 2.000mg/dia e etambutol 1.600mg/ /dia). As lesões imagiológicas estão descritas nas Figuras 1 a 3. Paralelamente iniciou o estudo osteoarticular.

Ao exame objectivo a doente apresentava todos os movimentos limitados e dolorosos da coxo-femoral esquerda e palpação dolorosa da sacroilíaca esquerda. Analiticamente apresentava elevação dos parâmetros de fase aguda [VS de 50 mm na 1ª hora (N< 20 mm na 1ª hora) e PCR de 4,75 mg/dl (N< 0,5 mg/dl)], anemia normocítica normocrómica (Hg de 10,8g/dL) e anticorpos VIH1 e VIH2 negativos. Na radiologia convencional da bacia, as articulações coxo-femorais não apresentavam alterações, observando-se discreta esclerose subcondral e irregularidade da entrelinha articular da articulação sacroilíaca esquerda e lesões osteolíticas localizadas ao ilíaco (Figura 4). A TAC da bacia revelou «…abcesso com atingimento do músculo

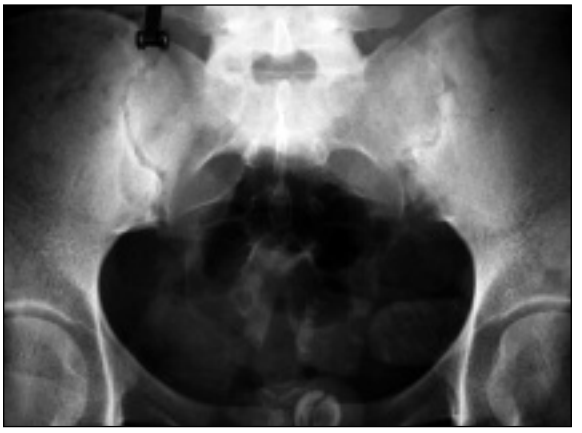


Figura 4. Rx da bacia AP – discreta esclerose subcondral e irregularidade da entre-linha articular da articulação sacro-ilíaca esquerda e lesões osteolíticas localizadas ao ilíaco.

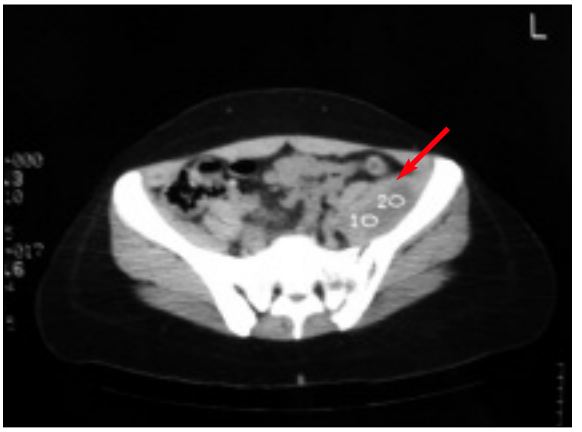


Figura 5. TAC da bacia – abcesso do músculo ilíaco esquerdo.

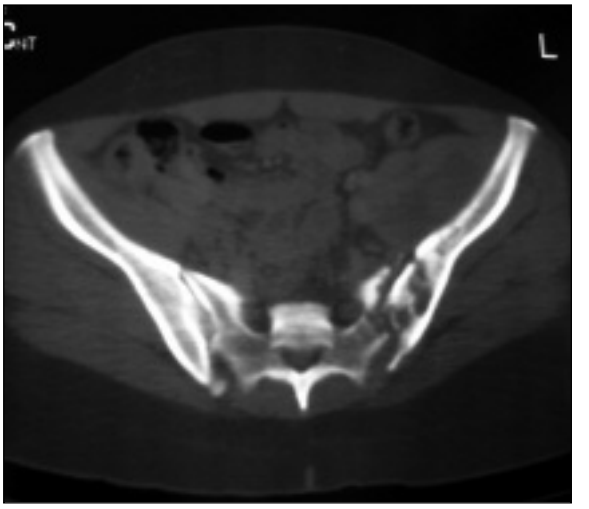


Figura 6. TAC da bacia – no osso ilíaco observam-se áreas de osteólise, uma delas mostrando um pequeno sequestro no interior.

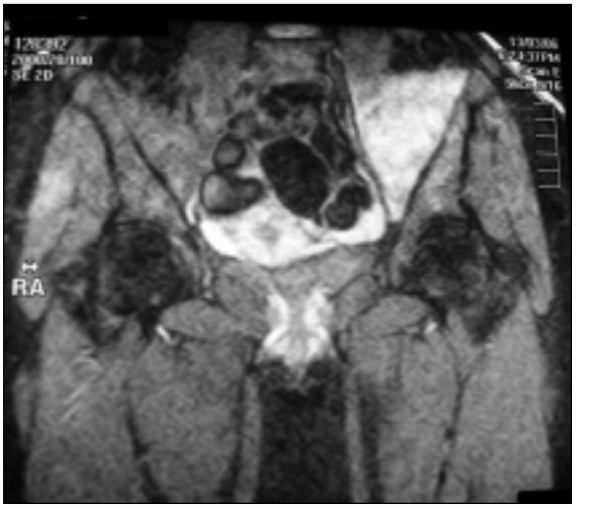


Figura 7. RMN da bacia – alterações da intensidade de sinal no osso ilíaco esquerdo, estendendo-se até à articulação sacro-ilíaca homolateral, envolvendo a asa esquerda do sacro.

ilíaco esquerdo e áreas de osteólise no osso ilíaco» (Figuras 5 e 6) e a RMN evidenciou «…espessamento do músculo ilíaco esquerdo e alterações da intensidade de sinal no osso ilíaco adjacente, estendendo-se até à articulação sacro-ilíaca homolateral» (Figura 7).

Foi ponderada intervenção cirúrgica para drenagem que foi considerada desnecessária atendendo às dimensões do abcesso.

A doente teve alta sendo orientada para o Centro de Diagnóstico Pneumológico (CDP) da área de residência e para a consulta de Reumatologia.

Durante o seguimento na consulta, sob terapêutica antituberculosa (isoniazida, rifampicina, pirazinamida, etambutol) apresentou melhoria clínica significativa regressando à actividade profissional. Houve normalização do hemograma e dos parâmetros laboratoriais de fase aguda (VS de 16 mm na 1ª hora e PCR < 0,5mg/dl). O controlo imagiológico por TAC mostrou resolução do abcesso do músculo ilíaco esquerdo mantendo contudo várias sequelas ósseas (Figuras 8 a 10).

Discussão

A tuberculose osteoarticular, embora não tão fre-

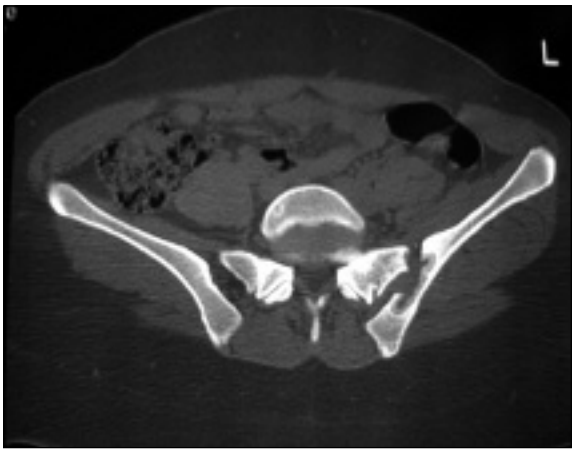


Figura 8. TAC da bacia – erosões osteolíticas na asa do ilíaco esquerdo.

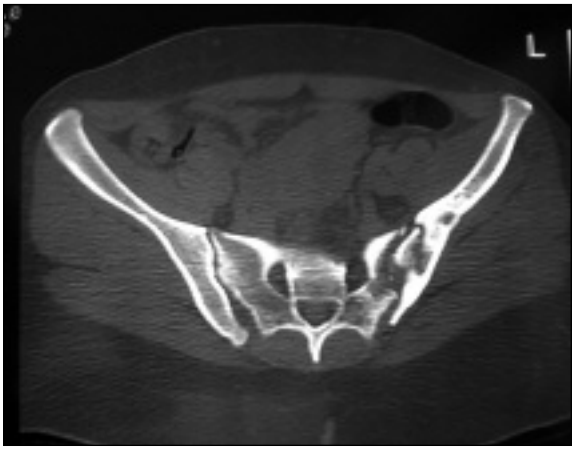


Figura 9. TAC da bacia – sinais de reabsorção óssea na vertente sagrada, junto ás alterações que atingem o sacro, com perda de definição da superfície articular.

quente como a pulmonar, continua a afectar adultos e a deixar sequelas limitantes e/ou deformantes devido ao atraso no diagnóstico e início da terapêutica.[11] A suspeita precoce e a rápida investigação clínica podem alterar radicalmente o curso da doença.

Apenas 30% dos doentes com tuberculose extrapulmonar apresentam evidência radiológica de tuberculose pulmonar. Tipicamente, existe um período longo (meses a anos) latente entre o primeiro episódio de infecção pulmonar e o desenvolvimento do envolvimento osteoarticular.[12] No nosso caso, o diagnóstico foi quase simultâneo. Provavelmente, o atraso no diagnóstico e na instituição da terapêutica para a tuberculose pulmonar favoreceram a infecção osteoarticular pelo *M. tuberculosis*.

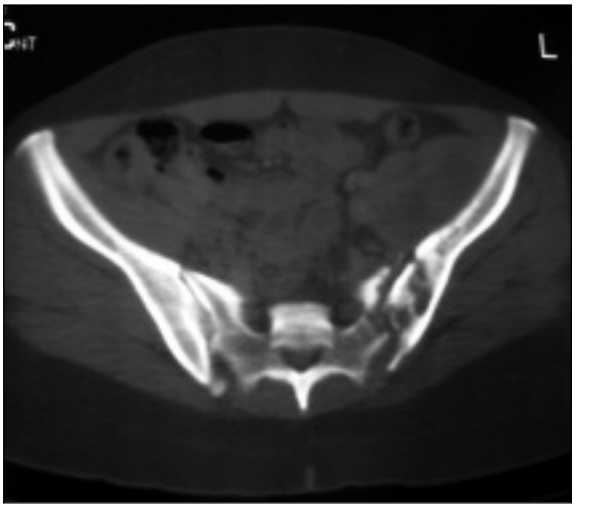


Figura 10. TAC da bacia – fragmento ósseo destacado da porção do ilíaco adjacente à articulação sacro-ilíaca esquerda.

Para evitar o atraso no diagnóstico, a tuberculose deve estar presente na lista de diagnósticos diferenciais de patologia osteoarticular uma vez que pode surgir em diferentes órgãos, particularmente em doentes provenientes de regiões endémicas, como o nosso país.

A apresentação clínica e radiológica da tuberculose osteoarticular em doentes oriundos de áreas endémicas difere dos de áreas não endémicas.[13] Os doentes de áreas endémicas apresentam uma maior incidência de envolvimento ósseo multifocal. Os achados radiológicos característicos são: reacção do periósteo, esclerose óssea e destruição óssea severa. No entanto, pode não ser observada qualquer alteração radiológica se o processo infeccioso for diagnosticado precocemente. A TAC é útil na detecção da destruição óssea, de massas nos tecidos moles adjacentes e ainda para drenagem de abcessos ou realização de biópsias percutâneas.[14] A RMN evidencia a extensão do envolvimento extra-esquelético, particularmente no caso de compromisso do canal vertebral e do espaço epidural.[13] Tanto a TAC como a RMN podem ser úteis no seguimento do doente avaliando a resposta à terapêutica.[15]

O tratamento da tuberculose osteoarticular é altamente eficaz usando os mesmos regimes recomendados para a tuberculose pulmonar. As recomendações terapêuticas incluem a associação da isoniazida, rifampicina, pirazinamida e etambutol durante 12 a 18 meses.[16]

O tratamento cirúrgico encontra-se reservado

para algumas situações clínicas específicas como a presença de alterações neurológicas, nos casos de cifose com instabilidade da coluna vertebral ou grande angulação; disseminação local da infecção e aspiração ou drenagem de abcessos.[17]

Nos casos de tuberculose da articulação sacroiliaca, Kim *et al* recomendam artrodese da articulação sacroilíaca quando existe grande destruição articular com instabilidade e presença de abcessos.[18]

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EULAR 2008

França, Paris
11-14 de Junho de 2008

SÍNDROME HEMOFAGOCÍTICA: UMA APRESENTAÇÃO INICIAL DO LÚPUS ERITEMATOSO SISTÊMICO

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Josué da Costa Arcoverde,** Loyana Pinheiro Costa,** Igor Denizarde Bacelar Marques,**
Gevina da Silva Pinheiro,*** Maria do Socorro Teixeira Almeida Moreira***

Resumo

A Síndrome Hemofagocítica é uma condição clínica caracterizada pela ativação de macrófagos e histiócitos com intensa hemofagocitose na medula óssea e em outros sistemas reticulo-endoteliais, levando à fagocitose de eritrócitos, leucócitos, plaquetas e seus precursores. A hemofagocitose pode estar associada a infecções, malignidades, doenças auto-imunes, drogas e outras situações. Descrevemos o caso de uma paciente, 23 anos, previamente saudável, que desenvolveu hemofagocitose ao mesmo tempo em que preencheu os critérios diagnósticos necessários para lúpus eritematoso sistêmico. Este caso é intrigante porque a síndrome hemofagocítica relacionada ao lúpus é uma entidade rara e potencialmente fatal, desafiante nos seus diagnósticos diferenciais e que requer intervenção terapêutica urgente. Há poucos casos relatados na literatura, sendo ainda necessário maior acúmulo de casos a fim de melhor compreender os aspectos clínicos, causas, mecanismos imunopatogênicos, critérios diagnósticos e tratamento desta síndrome, aqui sucintamente revisados.

Palavras-Chave: Síndrome Hemofagocítica; Lúpus Eritematoso Sistêmico.

Abstract

Hemophagocytic Syndrome is a clinical condition characterized by the activation of either macrophages or histiocytes with a prominent hemophagocytosis feature in the bone marrow and other reticuloendothelial systems. It leads to the phagocytosis of erythrocytes, leukocytes, platelets, and their precursors. The presence of hemophagocytosis can be associated to infections, malignancies, autoimmune diseases, drugs and a variety of other medical conditions. We report a case of a previously healthy 23 year-old woman that developed hemophagocytosis at the same time that she fulfilled diagnostic criteria for systemic lupus erythematosus. Lupus-related hemophagocytic syndrome is a rare and potentially fatal entity. It offers significant differential diagnosis challenges and requires urgent therapeutic intervention. There are only few cases reported in the literature. In this article, we briefly reviewed what is currently known about this syndrome. However, much is still needed in order to better understand its causes, all the immunopathogenic mechanisms, as well as its clinical aspects.

Keywords: Hemophagocytic Syndrome; Systemic Lupus Erythematosus.

Introdução

A Síndrome Hemofagocítica (HPS), também conhecida como linfohistiocitose hemofagocítica, é uma condição clínico-patológica caracterizada pela ativação de histiócitos na medula óssea e em outros sistemas retículo-endoteliais, levando à fagocitose de eritrócitos, leucócitos, plaquetas, e seus precursores. Esta entidade é mais freqüentemente descrita como complicação de infecção ou neoplasias malignas. Manifesta-se geralmente por febre elevada, hepatoesplenomegalia, linfadenopatia, perda de peso, pancitopenia, disfunção hepática, hiperferritinemia e coagulação intravascular dis-

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seminada.[1] Nos últimos anos, a associação com doenças auto-imunes tem sido reportada, incluindo o lúpus eritematoso sistêmico (LES).

Descrevemos o caso de uma paciente, previamente saudável, que desenvolveu HPS na mesma altura em que foi efectuado o diagnóstico de LES segundo o Colégio Americano de Reumatologia. Este caso é intrigante porque a síndrome hemofagocítica relacionada ao lúpus é uma entidade rara e potencialmente fatal, desafiante nos seus diagnósticos diferenciais e que requer intervenção terapêutica urgente. Uma revisão sistemática na base de dados do *Medline* revelou poucos casos relatados na literatura,[1-24] sendo ainda necessário maior acúmulo de casos a fim de melhor compreender os aspectos clínicos, causas, mecanismos imunopatogênicos, critérios diagnósticos e tratamento desta síndrome.

Relato do Caso

Paciente de 23 anos, sexo feminino, foi admitida com queixas de mialgias intensas, febre alta intermitente, perda ponderal de 8kg, adinamia e tosse produtiva com secreção ferruginosa acompanhada de dor pleurítica. Referia início dos sintomas dois meses antes, ocasião em que foi internada em outro hospital apresentando, além dos sintomas mencionados, icterícia de esclera, alteração da coloração da urina e anemia persistente, já tendo recebido, até aquele momento, seis bolsas de concentrado de hemácias.

Ao exame clínico, apresentava-se com um mau estado geral, taquicárdica (FC 120bpm), hipocorada 2+/4+ e com claudicação da marcha. A palpação abdominal evidenciava hepatoesplenomegalia discreta (cerca de 2-3cm do rebordo costal) e havia dor à palpação de vários grupos musculares, incluindo os abdominais, da região anterior e posterior dos braços, antebraços e coxas, gemelar das pernas e região tenar e hipotenar das mãos. Um discreto *rash* cutâneo em região malar podia ser observado já na admissão, porém esse fato só veio a ser considerado para o diagnóstico com a evolução do quadro clínico, acentuação do *rash* malar e a realização de exames complementares. O restante do exame físico sem mais anormalidades.

A avaliação laboratorial na admissão revelou anemia normocrômica e normocítica [Hemotócrito 18,8% (38-53%), Hemoglobina 6,2g/dL (12.5--16g/dL), contagem de hemácias 2,12 x 10^6/mm^3], leucocitose global com desvio à esquerda (contagem total 11.300/ mm^3, 10% de bastões, 78% neutrófilos, 6% linfócitos) e plaquetas normais. Os exames bioquímicos evidenciaram aspartato transaminase 199 U/L (< 46 U/L), alanina transaminase 155 U/L (< 49 U/L), fosfocreatinoquinase (CPK) 460 U/L (22-198 U/L), desidrogenase lática (LDH) 398 U/L , Gama-glutamil-transpeptidase (γGT) 101 U/L (7-30 U/L), fosfatase alcalina 176 U/L (98-279 U/L), Proteínas totais 6,4g/ dL (6-8,2g/dL), Albumina 2,6 g/dL (3,5-5,4g/dL), Sódio (Na) 135 mmol/ /L (135-150mmol/L), Potássio (K) 3,3 mmol/ L (3,5--5,5 mmol/L). Uréia, creatinina, glicemia de jejum, reticulócitos e bilirrubinas dentro dos valores de referência. Exame de urina tipo II tinha aspecto turvo, piócitos 15p/c, albumina 2+, vários cilindros granulosos, hematúria + e hemácias 4p/c. A proteinúria foi de 643mg/24h. Coombs direto positivo e haptoglobina indosável. Urocultura negativa. Ultrassonografia abdominal demonstrou hepatoesplenomegalia. Teste tuberculínico não reactivo (enduração de 0 mm). ECG revelou taquicardia sinusal e alterações difusas de repolarizacão ventricular. Ecocardiograma transtorácico demonstrou insuficiência mitral leve.

Foi instituída antibioticoterapia venosa sistêmica com ceftriaxone e oxacilina sem melhora clínica significativa. Após duas semanas, já havia recebido 7 bolsas de concentrado de hemácias, sem, no entanto, manter níveis hematimétricos aceitáveis. Iniciou-se, então, prednisolona oral 1mg/kg/ /dia, sem melhora clínica.

Cinco dias após o início da terapia com esteróide oral houve piora súbita do quadro clínico, com aparecimento de dispnéia, oligúria, taquicardia (FC 150 bpm) e hipotensão (70/40 mmHg). Nesse momento, a paciente já havia relatado alguns episódios de hemoptises, queixava-se de persistência da dor pleurítica e a ausculta pulmonar apresentava atrito pleural em terço inferior do hemitórax direito. Evoluiu com aparecimento de *rash* cutâneo generalizado principalmente em membros (Figura 1). A febre tornou-se persistente e diária com picos de 39,5ºC e houve surgimento de linfonodos de consistência elástica com 1cm no maior diâmetro cervicais e inguinais.

Leucócitos e plaquetas decaíram progressivamente, chegando a níveis de 1.000 células/mm^3 e 97000 células/mm^3, respectivamente. Apresentou elevação das escórias nitrogenadas, triglicérides 249 mg/dL (até 150 mg/dL), VLDL 50 mg/dL, ferritina 772 ng/mL (08-160 ng/mL), CPK 2004 U/L,

LDH 733 U/L. Sorologias para dengue, vírus da hepatite A, B e C, herpes tipo 1 e 2, citomegalovírus, Epstein-Barr, HIV 1 e 2, leptospirose foram IgM negativas. VDRL não reagente. Eletroforese de proteínas e hemoglobinas foram normais. Fibrinogênio plasmático 293 mg/dl (200-450 mg/dL). CH50 (Atividade hemolítica total do complemento) 88 µ/ /CAE (≥ 60 µ/CAE). Radiografia de tórax revelou infiltrado intersticial difuso.

A associação das alterações da ausculta pulmonar (atrito pleural) com dor pleurítica, *rash* cutâneo malar, pancitopenia, cilindrúria e proteinúria em paciente jovem do sexo feminino fortaleceram a suspeita de doença auto-imune, especialmente o LES. No entanto, a ausência de diagnóstico definitivo associado à piora da condição clínica da paciente apesar da antibioticoterapia e a incompatibilidade persistente dos testes para transfusão de concentrados de hemácias nos levaram a iniciar a pulsoterapia com metilprednisolona 1g por dia durante 3 dias ao mesmo tempo em que foi realizado um aspirado de medula óssea e solicitado uma pesquisa de anticorpos antinucleares. A resposta foi dramática. Já no segundo dia da pulsoterapia, os níveis de hemoglobina aumentaram e mantiveram-se estáveis até a alta hospitalar (11,5g/dL). A contagem de plaquetas e leucócitos também se normalizaram um dia após o término da terapia. O aspirado de medula óssea evidenciou hiperplasia histiocitóide com intensa hemofagocitose (Figuras 2 e 3), a pesquisa dos anticorpos antinucleares em células Hep-2 foi positiva com titulação 1/320 e padrão pontilhado, anticorpo anti-ENA total 1/512 e anticorpo anti-nRNP reagente. Anticorpos anti-SM, anti-DNA dupla hélice, anti-RO e anti-LA não reagentes.

A paciente recebeu alta uma semana após o término da pulsoterapia em boas condições clínicas, sem visceromegalias ou linfonodos palpáveis, normalização das enzimas hepáticas, da função hepática e das 3 séries hematológicas.

Discussão

A HPS pode estar associada a infecções, malignidades, doenças auto-imunes, drogas e outras situações.[25] Critérios diagnósticos já foram propostos por Hentel *et al*,[26] Imashuku,[27] e Tsuda,[28] sendo o primeiro estabelecido em 1991 por membros da sociedade de Histiocitose (Quadro I). Esses critérios, no entanto, não parecem adequados para o

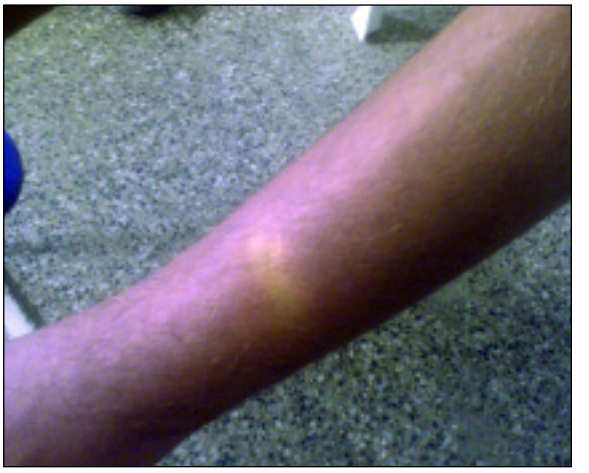

Figura 1. Digito-pressão em perna direita da paciente, demonstrando *rash* cutâneo intenso.

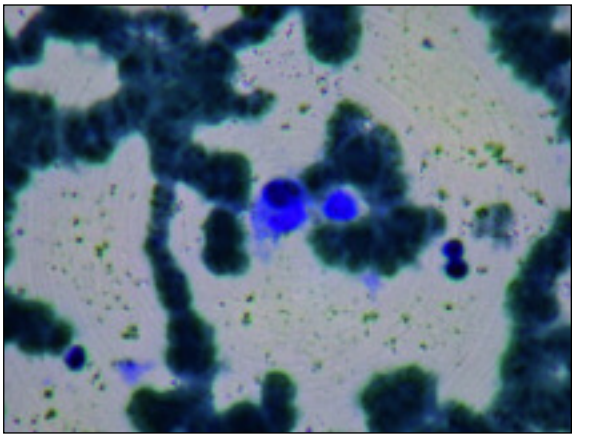

Figura 2. Aspirado de medula óssea demonstrando eritrofagocitose. (40x)

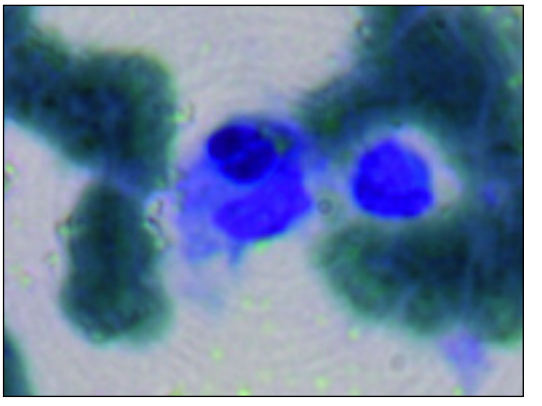

Figura 3. Histiócito em região central fagocitando hemácia. (40x)

Quadro I. Critérios diagnósticos para síndrome hemofagocítica propostos por membros da Sociedade de Histiocitose.

A. Critérios diagnósticos para HPS propostos por Hunter *et al*.:[26]
1. Critérios clínicos e laboratoriais
   Febre (duração ≥7 dias, com picos ≥38.5°C)
   Esplenomegalia (≥3 cm abaixo do rebordo costal)
   Citopenia (afetando ≥2 de 3 linhagens em sangue periférico e não causado por medula óssea hipocelular ou displásica): Leucócitos ≤1.0×10⁹/l, Hemoglobina ≤9 g/dl, Plaquetas ≤100×10⁹/l.
   Hipertrigliceridemia e/ou hipofibrinogenemia
   Elevação do Triglicerídeo ≥2.0 mmol/l ou ≥3 desvios-padrão do valor normal para idade; Fibrinogênio ≤1.5 g/l ou ≤3 desvios-padrão.
2. Critério Histopatológico
   Hemofagocitose na medula óssea ou baço ou linfonodos.
   Não evidência de malignidade.

B. Critérios diagnósticos para HPS, incluindo causas secundárias, propostos por Imashuku:[27]
1. Critério clínico e laboratorial
   Febre (duração ≥7 dias, com picos ≥38.5°C)
   Citopenia (afetando ≥2 of 3 linhagens em sangue periférico e não causado por medula óssea hipocelular ou displásica): Leucócitos ≤1.0×10⁹/l, Hemoglobina ≤9 g/dl, Plaquetas ≤100×10⁹/l.
   Hiperferritinemia e/ou Hiper-LDH-nemia
   Ferritina ≥3 Desvios-padrão do valor normal para idade, geralmente ≥1,000 ng/ml
   LDH ≥3 Desvios-padrão do valor normal para idade, geralmente ≥1,000 UI/l.
2. Critério Histopatológico
   Hemofagocitose na medula óssea ou baço ou linfonodos.
   Linfócitos granulares largos, maturos e imaturos, são freqüentemente aumentados em número.

C. Critérios diagnósticos para HPS propostos por Tsuda:[28]
1. Febre alta por mais de uma semana.
2. Citopenia não esclarecida progressiva afetando pelo menos duas linhagens celulares.
3. Medula óssea mostrando histiócitos maduros ≥3% ou 2,500 celulas/ L com hemofagocitose proeminente e/ou hemofagocitose em baço ou linfonodos.

O diagnóstico de HPS requer o preenchimento de todos os critérios acima.
A procura por história familiar, início de infecção, malignidades e estados imunossupressivos deve se processar.

diagnóstico de HPS quando a mesma está associada a doenças auto-imunes.[25]

No caso relatado, a febre perdurou por mais de uma semana com picos de 39,5ºC, acompanhada de esplenomegalia a 3cm do rebordo costal, pancitopenia, elevação dos níveis de LDH, hipertrigliceridemia, hiperferritinemia e hemofagocitose medular sem evidências de malignidade. Preenchia os critérios diagnósticos dos 3 autores mencionados, excepto a hipertrigliceridémia (valores elevados, mas não suficientes para ser cumprido o critério de Henter *et al*). Outros achados clínicos e laboratoriais são consistentes com o diagnóstico de HPS como sintomas cerebromeníngeos, edema, convulsões, linfonodomegalias, *rash* e anormalidades das enzimas hepáticas,[26] sendo os últimos 3 também presentes no referido caso.

Risdall *et al* fizeram a primeira descrição, em 1979, a partir de 19 pacientes com infecção viral ativa, de uma síndrome clínica envolvendo hiperplasia histiocitária, hemofagocitose e pancitopenia, a qual foi posteriormente reconhecida como sendo a síndrome hemofagocítica.[29] De fato, agentes infecciosos englobam principalmente os vírus, notadamente o Epstein-Barr (EBV) e o citomegalovírus (CMV). Associações com vírus da dengue, bastante incidente em nosso meio, herpes simplex, HIV, parvovírus B19, hepatites virais, assim

Quadro II. Critérios diagnósticos para síndrome hemofagocítica de causa auto-imune propostos por Kumakura[25]

1. Citopenia (acometimento de 2 das 3 linhagens em sangue periférico e não causado por aplasia ou displasia de medula óssea).
2. Hemofagocitose histiocitica na medula óssea ou em outros sistemas reticuloendoteliais incluindo baço, fígado ou linfonodos.
3. Ocorrência de hemofagocitose na fase ativa da doença auto-imune.
4. Exclusão de outras síndromes hemofagocíticas reacionais, tais como as causadas por vírus, malignidades.

Nota:

Às vezes se desenvolvem auto-anticorpos contra células hematopoiéticas.
Febre alta, hiperferritinemia e hiper-LDH-nemia não são absolutamente necessários.

como bactérias, fungos e protozoários, também são conhecidas. Além disso, as manifestações da própria síndrome podem ser confundidas com sepse grave bacteriana ou leptospirose.[30,31] No caso da paciente, o quadro clínico inicial foi compatível com leptospirose, e a evolução desfavorável com hipotensão severa, taquicardia associada a neutropenia grave levantou a possibilidade de sepse bacteriana. O quadro miálgico com espastamento gemelar e a elevação marcante da CPK chamaram bastante atenção, o que, associado à história inicial de alteração da coloração da urina, icterícia de esclera e epidemiologia compatível levantaram fortemente a suspeita de leptospirose. Por outro lado, a miopatia ocorre em mais de 50% dos pacientes com LES e está associada à elevação da CPK, fraqueza muscular proximal e presença de anticorpos antiribonucleoproteína (anti-nRNP),[32] sendo este anticorpo reagente na paciente e a possível explicação para a exuberância dos achados do sistema musculoesquelético. As sorologias IgM negativas para EBV, CMV, HIV, herpes tipo 1 e 2, leptospirose, dengue, além da negativação das culturas, ausência de resposta a agentes antimicrobianos associada a resposta dramática após pulsoterapia com metilprednisolona nos permitiram investigar outras possibilidades que não as infecciosas.

O conhecimento da síndrome hemofagocítica associada a auto-imunidade tem gradualmente crescido, e relatos de casos têm surgido de forma mais consistente nos últimos anos. Várias desordens auto-imunes estão documentadas: LES, síndrome de Sjögren, esclerose sistêmica progressiva, artrite reumatóide, vasculites, sarcoidose e mais recentemente a espondilite anquilosante.[2,33,34,35] Kumakura *et al* propuseram, então, em 2004, critérios para o diagnóstico desta nova entidade nosológica: síndrome hemofagocítica associada à auto-imunidade[25] (Quadro II).

Nossa paciente se enquadrava nos critérios diagnósticos de Kumakura *et al* e também preenchia 5 critérios do Colégio Americano de Reumatologia[36] para o diagnóstico de LES (*rash* malar, nefrite evidenciada tanto por cilindrúria quanto por proteínuria maior que 500 mg em 24h, FAN, pancitopenia, pleurite confirmada através da ausculta de atrito pleural localizado por médico experiente associada a dor pleurítica). A doença mista do tecido conjuntivo e a polimiosite são diagnósticos diferenciais possíveis, porém devemos lembrar que o tempo é um dos maiores aliados da reumatologia, e, neste momento, a paciente não apresentava critérios suficientes para o diagnóstico destas condições: ausência de queixas de fraqueza muscular proximal (quadro eminentemente miálgico), fenômeno de Raynaud, acroesclerose, mãos edemaciadas ou sinovite. Não apresentava evidências laboratoriais de infecções ou malignidades, no entanto também não respondeu a corticoterapia oral de 60 mg/dia de prednisolona feita inicialmente. Apesar da impossibilidade de se descartar completamente uma infecção viral subjacente, presume-se que o surgimento do próprio lúpus desencadeou a ativação macrofágica.

Os mecanismos patogênicos propostos, até o momento, da HPS no LES,[1-3] os quais não são mutuamente excludentes, são: 1) Fagocitose de células hematopoieticas mediada por anticorpos; 2) Deposição de imunocomplexos nas células hematopoiéticas da medula óssea e 3) Secreção exagerada de citocinas (IL-1, IL-6, INF-gama, TNF-α) por ativação descontrolada primária de células T. Neste caso, foram observados níveis de CH50 dentro

dos valores de referência (exame termolábil e passível de imprecisões). Vale ressaltar que, conforme proposto por Kazuma *et al,*[14] a produção em grande quantidade de citocinas inflamatórias pelo próprio LES e pela HPS pode levar à permeabilidade vascular aumentada e síndrome do desconforto respiratório agudo, o que pode explicar o achado radiológico de infiltrado intersticial difuso com melhora após pulsoterapia em nosso caso. A hipertrigliceridemia observada, por outro lado, pode estar relacionada com a diminuição da atividade lipolítica, o que pode ser induzida por citocinas, como a IL-1 e TNF-α.[35] O mecanismo imunopatogênico parece ser heterogêneo e ainda não está completamente esclarecido.[1,17,25,38]

Um tratamento padrão para HPS ainda não foi estabelecido, devendo ser individualizado de acordo com as diferentes situações clínicas e doenças de base visto que, por ser entidade rara, não existem estudos clínicos controlados.[24,25,30,39] Redução das doses de drogas imunossupressoras quando em uso e a administração de agentes antimicrobianos são feitos nos casos de relação com infecção.[24,30] Nas HPS associadas a doenças auto-imunes, a terapêutica deve ser direcionada para a doença de base, bem como para o bloqueio da produção de auto-anticorpos e citocinas. Altas doses de prednisolona ou a pulsoterapia com metilprednisolona têm sido escolhas com resultados satisfatórios em diversos relatos e série de casos.[3,16,19,25,38,40] Nos casos refratários ou severos, a pulsoterapia com ciclofosfamida tem sido realizada.[25] Alguns estudos reportaram o uso de tacrolimus, ciclosporina, imunoglobulinas intravenosa, plamaférese, etoposídeo, e outros agentes citostáticos de acordo com a situação clínica e doença de base do paciente avaliado e obtiveram resultados variáveis.[2,5,7,11,15,16,25,39] Stephan *et al.*, em estudo retrospectivo recente, obteve remissão em 15 dos 21 pacientes com episódios de hemofagocitose relativa a desordens inflamatórias utilizando monoterapia com esteróides.[40] Não existem estudos prospectivos a respeito do assunto, sendo a variabilidade no tratamento entre os diversos relatos e série de casos freqüente. A impossibilidade de se descartar infecção torna a decisão de início de terapia imunossupressora crucial para a vida do doente, uma tarefa que exige no mínimo muita atenção, prática e sensibilidade clínica.

Por fim, aqui descrevemos um caso de HPS como manifestação inicial de LES que respondeu ao uso de metilprednisolona na forma de pulsoterapia. Pacientes febris com pancitopenia, especialmente aqueles com anemia refratária, devem ser avaliados com um aspirado de medula óssea imediatamente, pois acreditamos ser a HPS uma entidade ainda sub-diagnosticada uma vez que pode levar o paciente a óbito rapidamente sem que se possa realizar o devido diagnóstico. Em casos de hemofagocitose confirmados, há de se investigar causas infecciosas, neoplasias, bem como patologias imunológicas, devendo se iniciar prontamente a terapêutica.

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HIPERPARATIROIDISMO PRIMÁRIO COEXISTINDO COM MIELOMA MÚLTIPLO: ASSOCIAÇÃO E MANIFESTAÇÕES RARAS

Mónica Bogas,* Lúcia Costa,* Domingos Araújo*

Resumo

Doenças como o mieloma múltiplo e o hiperparatiroidismo primário são encontradas com alguma frequência em doentes de idade mais avançada. Apesar de muito rara, a coexistência de ambas num mesmo doente, já foi anteriormente descrita na literatura. A sobreposição de manifestações comuns às duas patologias, nomeadamente a hipercalcemia e a osteopenia, pode dificultar o diagnóstico, tornando-o um desafio. Os autores apresentam o caso clínico de uma doente do sexo feminino em que a investigação de quadro de hipercalcemia associada a manifestações músculo-esqueléticas permitiu o diagnóstico simultâneo de mieloma múltiplo e hiperparatiroidismo primário. Com este caso clínico salientam-se ainda manifestações clínicas e radiológicas destas doenças, actualmente pouco observadas, associadas ao longo tempo de evolução clínica.

Palavras-Chave: Mieloma Múltiplo; Hiperparatiroidismo Primário; Hipercalcemia

Abstract

Diseases such as multiple myeloma and primary hyperparathyroidism are occasionally found in the elderly. Although very rare, the coexistence of both in the same patient has been described in the literature. The coincidence of manifestations of both diseases, namely hypercalcemia and osteopenia, may difficult the diagnosis, turning it into a challenge. The authors report a case of a female patient in whom the investigation of hypercalcemia and musculoskeletal manifestations led to a simultaneous diagnosis of multiple myeloma and primary hyperparathyroidism. With this case, they also emphasise clinical and radiological manifestations of these diseases, nowadays rarely observed, and related to the long time clinical evolution.

Keywords: Multiple Myeloma; Primary Hyperparathyroidism; Hypercalcemia

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Introdução

A hipercalcemia é uma manifestação comum a várias doenças, sendo as causas mais frequentes, a patologia endócrina e a neoplásica.[1,2] Apesar de muito rara, a coexistência de hiperparatiroidismo primário (HPP) com mieloma múltiplo (MM), já foi descrita e não parece ser apenas uma coincidência, podendo ser considerados, na sua fisiopatologia, factores humorais comuns.[3,4] A sobreposição de manifestações, comuns às duas patologias, pode dificultar o diagnóstico. Os autores apresentam um caso clínico onde as duas patologias foram diagnosticadas na mesma doente no decurso da investigação de um quadro clínico, com vários anos de evolução, de manifestações músculo-esqueléticas e hipercalcemia.

Caso clínico

Doente de 72 anos, sexo feminino, portuguesa e de raça caucasiana, observada na consulta de reumatologia referindo poliartralgias de ritmo misto de início há muitos anos, envolvendo predominantemente mãos, punhos, cotovelos, joelhos e tibio-társicas de forma simétrica, por vezes com sinais inflamatórios das mãos e punhos, acrocianose nas mãos, anorexia e fraqueza muscular generalizada. À observação apresentava baixa estatura, acentuação da cifose dorsal, atrofia tenar, afilamento, esclerodactilia e aspecto de retracção tendinosa dos dedos, lesões sugestivas de vasculite nas polpas digitais e esboço de tenossinovite dos extensores das

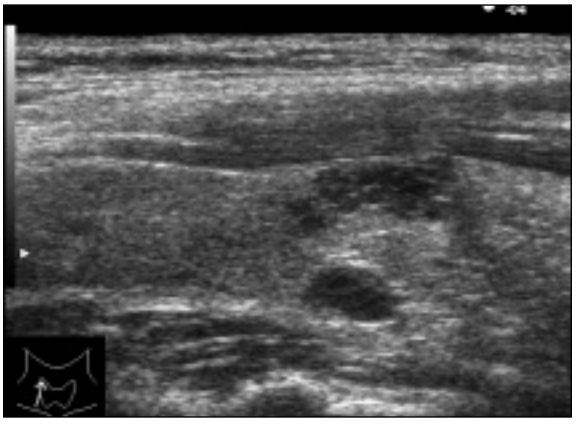

Figura 1. Ecografia do pescoço revelando nódulo sólido compatível com adenoma da paratiroideia.

mãos, nódulos subcutâneos de consistência pétrea nas superfícies de extensão dos cotovelos, valgismo e alargamento de consistência óssea dos joelhos e atrofia quadricipital. No exame geral constatou-se hipertensão arterial. Não eram palpáveis adeno ou organomegálias e a auscultação cardio-pulmonar, o exame da cabeça, pescoço e abdómen eram normais. A doente referia antecedentes de osteoporose e estava medicada com um anti-inflamatório, cálcio e um bifosfonato.

No estudo laboratorial apresentava anemia normocítica normocrómica (Hb-10,9 mg/dl VGM-90,4 CHCM-33,2), hipercalcemia (13,66 mg/dl), hipofosfatemia (2,18 mg/dl), hiperuricemia (7,26mg/ /dl), proteínograma eletroforético com proteínas totais de 8,0 g/dl e pico monoclonal na fracção gamma 2,10 g/dl (28,8%), doseamento de IgG elevado (2930 mg/dl) com IgA e IgM normais, β2-microglobulina elevada (2,54 ng/dl), sendo a proteína C reactiva e a velocidade de sedimentação globular normais. Os factores reumatóides e o estudo para despiste de conectivites foi negativo. Os restantes parâmetros laboratoriais nomeadamente, ferro sérico, ferritina, função renal, provas hepáticas, FA, urina II, proteinúria e calciúria das 24 h, eram normais.

Atendendo ao quadro clínico e às alterações laboratoriais encontradas impunha-se o estudo de causas frequentes de hipercalcemia, como o hiperparatiroidismo e neoplasias malignas.

O doseamento sérico da paratormona estava elevado (PTH-106 pg/ml) e, posteriormente, uma ecografia do pescoço mostrou um nódulo sólido, de contornos bem definidos, adjacente à tiróide, compatível com adenoma de paratiróide (Fig.1). A

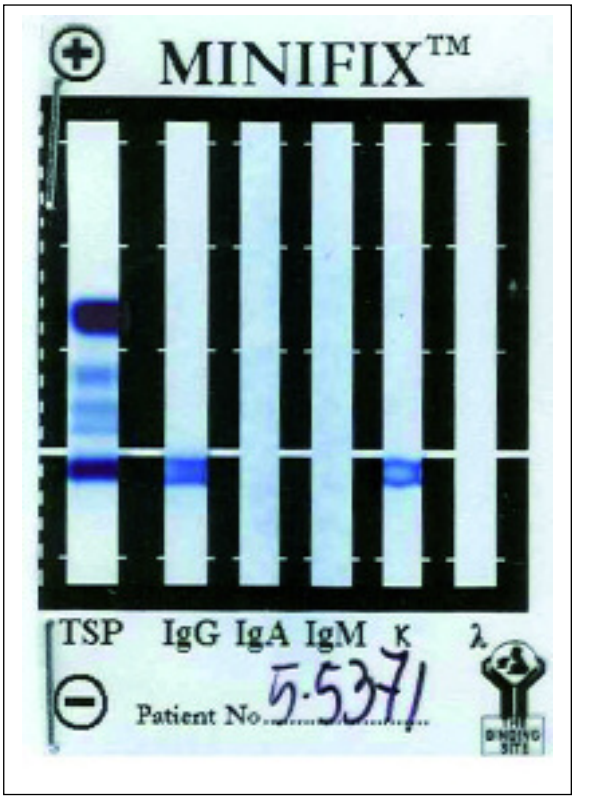

Figura 2. Bandas monoclonais IgG k na electroforese sérica.

presença de proteinúria de Bence-Jones e uma banda de precipitação do tipo monoclonal no arco da IgG e de cadeias leves Kappa na imunoelectroforese do sangue e urina (Fig.2) motivou a realização de medulograma e biopsia óssea cujo exame histológico mostrou infiltração medular intersticial por plasmócitos, formando «ninhos» e em cujo estudo imunocitoquímico se constatou positividade para cadeias leve Kappa nos infiltrados de plasmócitos, compatíveis com mieloma múltiplo/ /plasmocitoma (Fig 3 e 4).

No estudo radiológico evidenciavam-se sinais de osteoporose fracturaria na coluna dorsal, calcificação do ligamento triangular do carpo e dos meniscos e algumas calcificações periarticulares nos cotovelos e mãos.

A densitometria óssea apresentava valores de T-*-score* de -4,44 na coluna lombar e de -2,93 no colo do fémur. Na TAC toraco-abdominal não foram detectadas quaisquer outras alterações.

A doente iniciou quimioterapia com melphalan 10 mg po/dia e prednisolona 60 mg po/dia durante 4 dias de 5/5 semanas para tratamento do mieloma múltiplo e, posteriormente, tratamento de

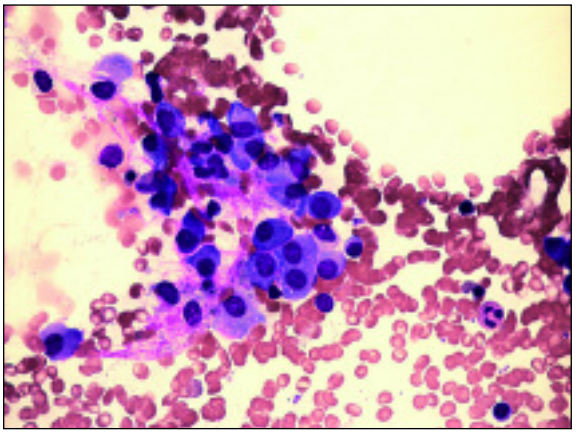

Figura 3. Ninhos de plasmocitos no medulograma.

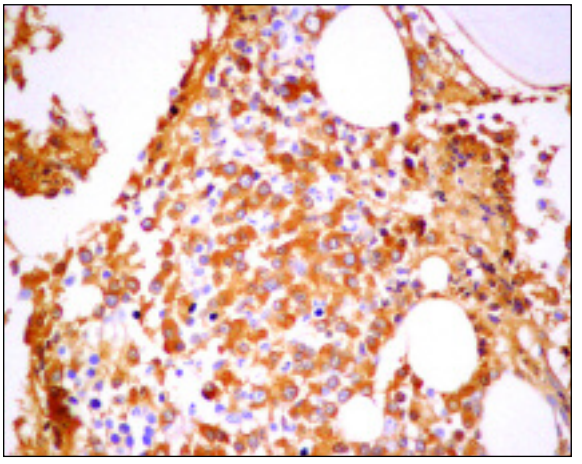

Figura 4. Imuno histoquímica da medula óssea revelando positividade para cadeias kappa.

consolidação com α-interferon 3 milhões unidades 3X/sem, durante 6 meses, obtendo-se a remissão. Recusou a cirurgia para paratiroidectomia.

Quatro anos depois do primeiro tratamento quimioterápico, verificou-se recidiva do mieloma múltiplo retomando terapêutica com ciclos de melphalan e prednisolona, não tendo sido possível obter de novo critérios de remissão. Manteve-se a vigilância desta doente na consulta verificando-se progressiva debilitação, sendo necessário frequentemente o seu internamento para redução dos níveis séricos do cálcio com pamidronato e, posteriormente, para tratamento de processos infecciosos associados a depressão medular. O cálcio sérico total máximo determinado foi de 16,9 mg/dl, mesmo sob terapêutica com pamidronato. Além de sintomas como anorexia, fraqueza muscular generalizada e disfagia, a doente nunca apresentou manifestações psíquicas, neurológicas e/ou electrocardiográficas associadas à hipercalcemia.

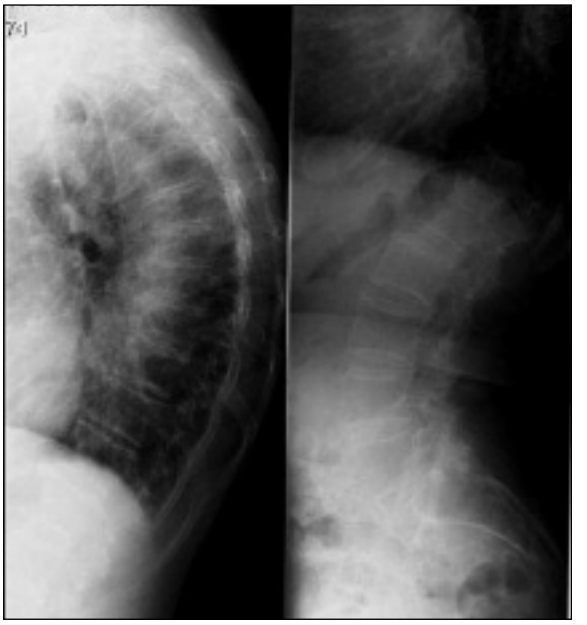

Figura 5. Osteopenia difusa e colapsos vertebrais condicionando aumento da cifose dorsal.

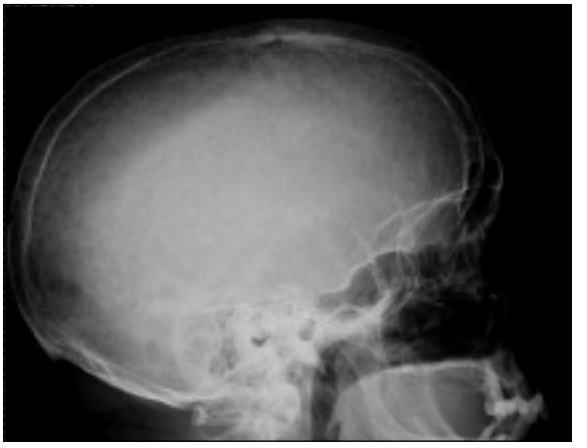

Figura 6. Lesões da calote craniana em «sal e pimenta».

Na última reavaliação radiográfica, foram evidentes, para além das fracturas vertebrais osteoporóticas e da osteopenia generalizada, erosões e sinais de reabsorção subperióstea das falanges das mãos, lesões em imagem «sal e pimenta» no crânio, condrocalcinose nos joelhos, sínfise púbica, articulações coxo-femorais e punhos e calcificações heterotópicas periarticulares nos joelhos e cotovelos (Figs 5 a 9).

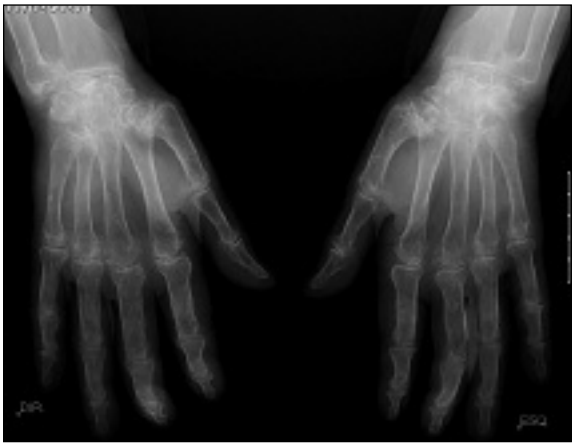

Figura 7. Reabsorção subperióstea das falanges e calcificação do ligamento triangular do carpo.

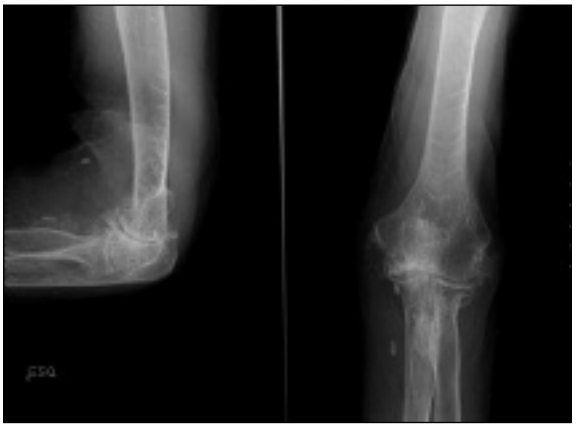

Figura 8. Calcificações periarticulares dos cotovelos.

A doente faleceu, no domicílio, na sequência de provável infecção respiratória.

Discussão

Esta doente representa um caso raro de associação de hiperparatiroidismo primário e mieloma múltiplo com manifestações clínicas e radiológicas presentemente pouco habituais.[5,6] Como actualmente a avaliação laboratorial do metabolismo fosfo-cálcico é mais acessível e faz parte da investigação inicial de algumas patologias, o diagnóstico de doenças associadas a hipercalcemia é agora mais precoce e com quadros clínicos menos floridos.[5]

Considera-se hipercalcemia quando o valor total de cálcio sérico é superior a 10,2 mg/dl (2,55mmol/L). O cálcio ionizado é uma medida mais precisa, sendo considerados normais os valores entre 4,48 e 4,92 mg/dl (1,12 a 1,23 mmol/l).[7,8] Como principais causas de hipercalcemia devem ser consideradas as neoplasias malignas e as doenças endócrinas, nomeadamente, o hiperparatiroidismo, o hipertiroidismo e a doença de Addison.[1,2] A hipercalcemia associada a malignidade pode resultar de tumores dependentes da proteína relacionada com a hormona paratiróide (PTHrP) ou outros factores humorais, ou de metastização óssea osteolítica.[8-12] Outras causas de hipercalcemia poderão ser consideradas: as doenças granulomatosas (sarcoidose, tuberculose), a imobilização prolongada e alguns medicamentos (tiazídas, lítio, intoxicação por vitamina A e D). O hiperparatiroidismo primário é considerado a principal causa de hipercalcemia no doente ambulatório e as neoplasias são a causa mais frequente de hipercalcemia nos doentes hospitalizados.[1,5,7,8]

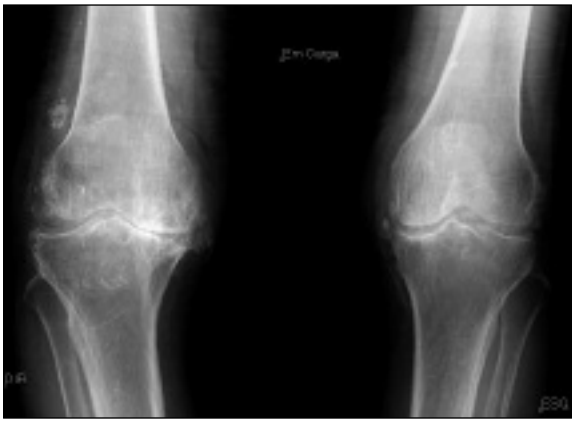

Figura 9. Calcificações meniscais e periarticulares dos joelhos.

No caso clínico descrito, a doente referia sintomatologia e apresentava sinais clínicos que fizeram pensar numa doença do tecido conjuntivo, nomeadamente manifestações que impunham o diagnóstico diferencial com a artrite reumatóide ou a esclerose sistémica. No entanto, a investigação inicial apontou para outra etiologia quando, ao verificar-se a presença de hipercalcemia, se incluiu, logo de início, quer o doseamento da hormona paratiroideia, quer a electroforese das proteínas no sangue, o que, com o estudo subsequente, permitiu o diagnóstico das duas patologias em simultâneo, hiperparatiroidismo primário (HPP) e mieloma múltiplo (MM).

Até ao momento, encontrámos 21 casos seme-

Quadro I. Hiperparatiroidismo primário e mieloma múltiplo: casos clínicos descritos na literatura

Nº	Referência	Idade	Sexo	Diagn. inicial	Ig/cadeia leve	Cálcio total (mg/dl)	Histologia paratiroide
13	Jackson,1979	45	F	MM	IgG lambda	17,1	Adenoma
14	Chisholm, 1981	80	M	HPP	Kappa	13,1	Adenoma
15	Francis, 1982	70	F	HPP	Lambda	11,6	Adenoma
16	Mundis, 1982	76	F	MM	IgG kappa	11,0	Adenoma
17	Stone, 1982	47	F	HPP+MM	IgA kappa	14,3	Adenoma
18	Hoelzer, 1984	51	F	HPP MM	IgA lambda	11,9	Adenoma
19	Dalgleish, 1984	59	F	MM	IgG lambda	NA	NA
20	Sarfati, 1985	62	F	MM	IgA kappa	16,5	Adenoma
21	Katayama, 1989	50	F	HPP	NA	NA	NA
22	Schneider, 1989	74	F	MM	IgG kappa	12,0	Adenoma
23	Romagnoli, 1990	70	F	HPP(MEN-1)	ND	ND	Adenoma
24	Rao, 1991	54	M	HPP+MM	IgG lambda	11,2	Adenoma
25	Rosen, 1992	81	M	MM	IgG kappa	13,4	ND
26	Toussirot, 1994	82	F	MM	Kappa	15,2	Hiperplasia
27	Goto, 1995	73	F	HPP	Kappa	13,2	Adenoma
28	Otsuka, 1997	77	F	MM	IgG lambda	12,5	Hiperplasia
29	Toh, 2000	71	M	MM	ND	12,0	ND
30	Sopena, 2004	77	F	HPP	ND	12,9	ND
31	Khandwala, 2004	72	F	HPP	ND	11,7	Adenoma
32	Patel, 2005	73	F	MM	IgG kappa	13,5	Adenoma
33	Fery-Blanco, 2007	68	F	MM	IgG kappa	11,3	Adenoma

ND – não determinado; NA – não acessível

lhantes na literatura, espalhados pelo mundo, sendo esta a primeira descrição em Portugal (Quadro I). Nestes casos, o mieloma múltiplo foi o diagnóstico inicial mais vezes descrito e mais frequentemente considerado como a causa de hipercalcemia. No entanto, nos vários casos descritos, a ausência de lesões líticas e a persistência da hipercalcemia após terapêutica apropriada, ajudava a revelar uma outra causa para a hipercalcemia. A idade média de diagnóstico foi aos 67 anos (min-45, max-82), sendo os doentes do sexo feminino em 17 casos (81%). O adenoma foi o diagnóstico histológico mais frequente do tumor paratiroideu e, a IgG kappa foram a imunoglobulina e a respectiva cadeia leve mais produzidas. No caso clínico apresentado, o diagnóstico das duas patologias foi simultâneo. No entanto, atendendo às manifestações radiológicas preponderantes e à ausência de lesões líticas, parece haver mais dados a favor de que o tumor paratiroideu possa ter surgido em primeiro lugar. Neste caso não foi efectuado o estudo histológico do tumor paratiroideu mas, pelas suas características, tratar-se-ia muito provavelmente de um adenoma solitário.

A incidência anual de hiperparatiroidismo primário é bastante superior à do mieloma múltiplo podendo o achado do último, no mesmo doente, ser considerado fortuito.[34-37] No entanto, a ocorrência de hiperparatiroidismo primário em associação com neoplasias linfoproliferativas ou carcinomas sólidos, como o do pulmão, mama, rim, próstata, tiróide e fígado, tem sido descrita na literatura embora a sua raridade tenha contribuído para a dificuldade em esclarecer os mecanismos etiopatogénicos subjacentes e para a limitação em definir orientações diagnósticas.[8,34,38]

A hipótese de que uma das doenças constitui o primeiro evento e que esta induz o aparecimento da outra tem sido colocada.[23] É possível que factores solúveis produzidos por um determinado tumor desencadeiem o crescimento de outras populações tumorais. Alguns estudos demonstraram que o cálcio actua como factor mitogénico capaz de estimular *in vitro* algumas linhas celulares o que poderia acontecer pelos elevados níveis séricos de cálcio associados ao hiperparatiroidismo

primário em relação à proliferação celular do mieloma múltiplo.[39] Por outro lado, as proteínas anómalas produzidas no mieloma múltiplo poder-se-iam ligar à própria glândula paratiroideia interferindo na síntese hormonal e/ou acoplarem-se às suas fracções circulantes ou bloquear os seus efeitos periféricos, estimulando secundariamente a paratiróide.[40]

Mais recentemente, na tentativa de explicar a ocorrência concomitante do hiperparatiroidismo primário e mieloma múltiplo no mesmo doente, foram referidas algumas citocinas comuns e com papel importante na remodelação óssea. Sabe-se que a hormona paratiroideia (PTH) actua, em parte, pela indução dos osteoblastos a produzir citocinas que estimulam a diferenciação e a actividade reabsortiva dos osteoclastos.[3,41-43] A IL-6 foi identificada como uma dessas citocinas que se sabe, simultaneamente, actuar como um potente factor de crescimento das células do mieloma e ser responsável pelas características reabsortivas ósseas desta doença.[4,44-46]

No HPP a secreção inapropriada elevada de PTH origina uma excessiva reabsorção de cálcio, fosfatúria e síntese de 1,25(OH)2 D3.[5] O diagnóstico de HPP é estabelecido quando estão presentes hipercalcemia e níveis séricos elevados de PTH.[1,5,7,47]

Nas descrições iniciais, os doentes com HPP apresentavam frequentemente envolvimento músculo-esquelético com tradução radiográfica e a doença era referida como tendo morbilidade e mortalidade elevadas.[5]

O envolvimento do esqueleto reflecte o aumento da actividade osteoclástica traduzido por uma desmineralização generalizada e osteoporose.[48] O compromisso músculo-esquelético resulta de: 1) fraqueza muscular proximal ou generalizada; 2) artropatia associada a reabsorção óssea, erosões por reabsorção subcondral ou subperióstea, deposição de cristais de pirofosfato de cálcio (10%), deposição de cristais de monourato de sódio e sinovite osteogénica (pequenas articulações das mãos, punhos, joelhos, pés, sacro-ilíacas e outras articulações do esqueleto vertebral); 3) doença óssea por reabsorção subperióstea com perda do contorno ou substância óssea com ou sem compromisso articular (falanges das mãos, extremidade distal das clavículas) ou reabsorção trabecular, particularmente nítida no crâneo (imagem em «sal e pimenta»); 4) envolvimento das estruturas periarticulares por calcificações heterotópicas, reabsorção subligamentar e subtendinosa; 5) tumores castanhos, únicos ou múltiplos, actualmente raros (ossos longos, face, pélvis).[1,5,7,49-52]

Além do envolvimento músculo-esquelético, o quadro clínico de HPP pode incluir manifestações associadas à hipercalcemia em si e/ ou a manifestações renais, pancreáticas, gastrointestinais, cardiovasculares e neuro-psiquiátricas.[1,5,7,53]

Contudo, actualmente, no momento do diagnóstico, os doentes estão, na maior parte dos casos, assintomáticos. Pode mesmo dizer-se que o quadro clínico do HPP tem vindo a alterar-se ao longo dos tempos, encontrando-se na literatura a terminologia de quadro clínico «clássico» e «moderno» de HPP.[5] Na maioria dos casos, os doentes apresentam elevação da concentração sérica de cálcio e da PTH.[54] Em 75-85% dos casos o HPP resulta de um adenoma na paratiróide.[5,48]

Para além da anemia e insuficiência renal, a astenia, a fraqueza muscular generalizada e as lesões líticas fazem também parte do quadro clínico de um doente com mieloma múltiplo.[55] Estas manifestações têm um forte valor diagnóstico se acompanhadas pela presença de mais de 10% de plasmócitos atípicos na medula óssea e de imunoglobulina monoclonal no soro ou cadeias leves na urina.[6] A hipercalcemia no contexto de mieloma múltiplo ocorre em 20 - 40% dos casos.[45] Tal como no caso descrito, apesar de raro, o mieloma múltiplo pode estar associado a oligo ou poliartrite erosiva de pequenas e médias articulações simulando a artrite reumatóide e a referência a vasculite cutânea associada a mieloma múltiplo, ou a outras neoplasias, pode também ser encontrada na literatura.[56,57] Quase todos os doentes com mieloma têm uma destruição óssea extensa que se pode apresentar como lesão localizada ou comprometer de forma difusa todo o esqueleto.[45] O compromisso ósseo parece resultar da estimulação por algumas citocinas (nomeadamente pela IL-6) do aumento da expressão pelos osteoblastos do ligando de RANK (RANKL) e da redução da produção de osteoprotegerina, o que origina a activação dos osteoclastos e respectiva reabsorção óssea.[45,58,59]

Conclusão

Apesar de a natureza da associação do hiperparatiroidismo e mieloma múltiplo se manter especulativa, têm sido considerados, na sua fisiopatologia, factores humorais comuns. O diagnóstico individual de qualquer uma destas doenças não ex-

clui a presença da outra e a falha no diagnóstico de uma pode resultar em morbilidade e mesmo em mortalidade. Ambas devem ser consideradas no diagnóstico diferencial de hipercalcemia, fazendo parte do seu estudo a realização de uma electroforese das proteínas e o doseamento da PTH.

As manifestações osteo-articulares do hiperparatiroidismo primário e do mieloma múltiplo são variadas, multifactoriais e, por vezes, comuns. A raridade desta associação e a sobreposição de algumas manifestações tornam este diagnóstico um desafio. O longo período de evolução no caso descrito, particularmente pela recusa da doente em ser submetida a cirurgia, possibilitou o desenvolvimento de alterações clínicas e lesões radiográficas actualmente pouco observadas na prática clínica.

Uma vez que a prevalência de HPP é superior à do MM, o despiste de gamapatia monoclonal poderá ser considerado em todos os doentes com o diagnóstico prévio de HPP.

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EXANTEMA EVANESCENTE

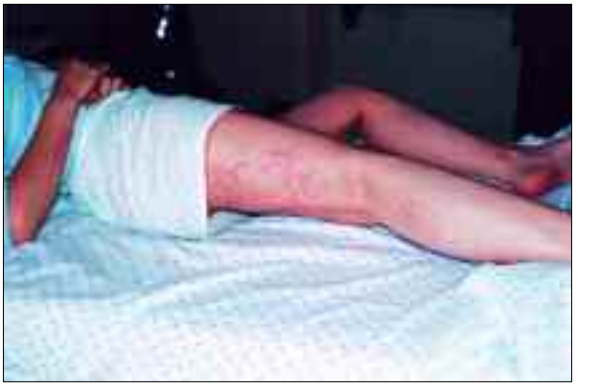
Maria João Saavedra,* Margarida Alexandre,** Armando Malcata*

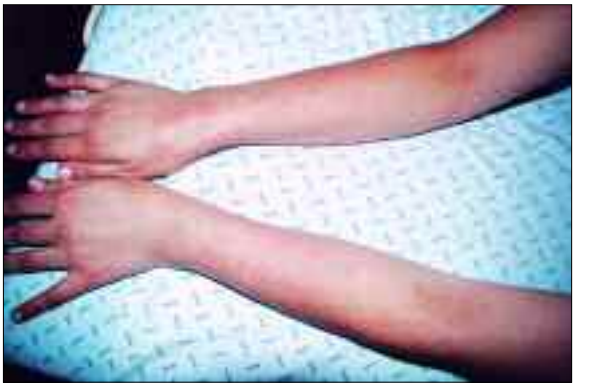
Doente do sexo feminino, 23 anos de idade, internada por quadro de exantema maculopapular evanescente, febre e artralgias de ritmo misto dos punhos, joelhos e pés, com três semanas de evolução.

Ao exame objectivo apresentava um a dois picos de febre diária (temperatura axilar >38,5º C), associados a exantema maculopapular evanescente, cor salmão, não pruriginoso do tronco, membros superiores e inferiores (Fig. 1-3); hiperemia da orofaringe e adenopatias cervicais e sub-mandibulares bilaterais, móveis e indolores à palpação; auscultação cardíaca: sopro sistólico grau II/VI no foco tricúspide; auscultação pulmonar sem alterações; exame abdominal normal; sem artrite periférica.

Do estudo complementar efectuado destacamos: leucocitose (19,000/µL) com neutrofilia (91,2%), elevação da velocidade de sedimentação (VS=85 mm 1ª H), da proteína C reactiva (PCR=28,1 mg/dL) e da ferritina (ferritina=4670 ng/mL), factor reumatóide (FR) e anticorpos-antinucleares (ANA) ausentes, fracções do complemento normais, hemo e uroculturas e serologias virais negativas, radiografias do tórax e osteoarticulares sem alterações significativas, ecografia abdominal normal e ecocardiograma com regurgitação tricúspide ligeira. Perante este quadro clínico estabeleceu-se o diagnóstico de doença de Still do adulto. Foi introduzida terapêutica com naproxeno e paracetamol, com melhoria discreta das artralgias, mas com manutenção dos picos febris e do exantema. Posteriormente foi iniciada corticoterapia oral com melhoria clínica e analítica. A terapêutica foi suspensa de forma progressiva e a doente mantém-se em remissão desde há três anos.

A doença de Still do adulto é uma doença rara de etiologia desconhecida. O exantema macular ou maculopapular, evanescente, cor salmão, do tronco e extremidades que acompanha os picos febris é característico desta doença. De acordo com os cri-





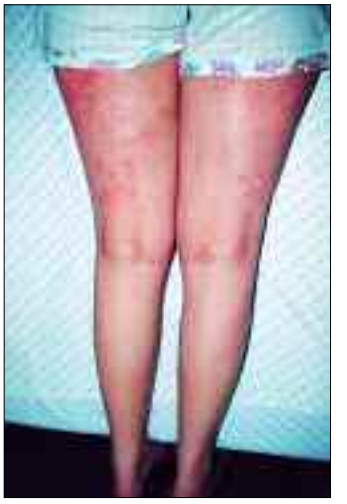


Fig. 1, 2 e 3. Exantema maculopapular evanescente, cor salmão dos membros superiores e inferiores

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**Serviço de Reumatologia do Centro Hospitalar da Cova da Beira, EPE

térios de diagnóstico de Yamaguchi, estabelece-se o diagnóstico de doença de Still se estiverem presentes 5 dos critérios abaixo indicados (sendo dois deles *major*). São critérios *major*: febre diária (39ºc ou superior) com pelo menos uma semana de evolução; artralgias ou artrite com pelo menos duas semanas de evolução; presença de exantema característico, nos picos febris; leucocitose (10.000/µL ou superior) com neutrofilia (80% ou superior). São critérios *minor*: presença de odinofagia; linfadenopatias; alteração das provas de função hepática; FR e ANA ausentes. A evolução desta doença é variável, podendo ser auto-limitada, com resolução completa dos sintomas; evoluir de forma intermitente com agudizações e períodos de remissão ou evoluir de forma crónica com artrite destrutiva. Os anti-inflamatórios não esteróides são a terapêutica de primeira linha; no entanto, muitos doentes necessitam de efectuar terapêutica com glucocorticóides para que haja uma melhoria clínica. Nos casos mais graves poderá haver necessidade de imunossupressores ou agentes biológicos.

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6th International Congress on Autoimmunity

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03-07 de Setembro 2008

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15th PRES

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